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Prevalence of Zoonotic Parasites and Diseases Among Illinois Raccoons

Federal Aid in Wildlife Restoration Project W-119-R-3

Final Report
July 1, 1992 through June 30, 1995

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PREVALENCE OF ZOONOTIC PARASITES AND DISEASES AMONG ILLINOIS RACCOONS (W-119-R-1-3)

Period Covered: July 1, 1992 through June 30, 1995

Submitted by: Laura L. Hungerford, DVM, MPH, PhD
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Job 1.1: Prevalence and rates of zoonoses and other diseases in sentinel, sick, and dead raccoons.

Objectives:

1. To determine the cause of death and general health status of each intact raccoon submitted for postmortem evaluation.
2. To investigate, using tissues and other samples, the cause of death and health status of raccoons necropsied by Illinois Department of Natural Resources (IDNR) personnel in the field and submitted to the University of Illinois.
3. To compile postmortem data on health and disease in rural and urban populations of raccoons.

Materials and Methods:

Data were collected from sentinel, sick, and dead raccoons submitted to the State of Illinois Diagnostic Laboratories, primarily the Diagnostic Laboratory at the University of Illinois and from specimens submitted to the diagnostic laboratory by IDNR personnel trained as described in Job 1.3. Each animal received, where appropriate, gross and microscopic postmortem evaluation, parasitologic (ectoparasites, endoparasites), virologic (rabies, pseudorabies, distemper), bacterial (leptospirosis, aerobic, anaerobic and mycotic culture), toxicologic (herbicides, insecticides), and other applicable diagnostic tests. Each raccoon necropsy yielded ten to twenty histopathologic slides for microscopic examination. Tissues were histopathologically evaluated by Dr. J. F. Zachary. Feces and gross endo- and ectoparasites were collected for parasitic identification. Unstained sections of brains were prepared and sent for immunohistochemical differentiation between *Toxoplasma* and *Sarcocystis* on selected animals. Fluorescent antibody tests were used to detect the presence of the distemper virus. Feces from raccoons were sent to electron microscopy lab for detection of parvo and corona virus particles. During the first year of the project, samples from raccoon brains were sent to the Illinois Department of Public Health in Springfield to test for rabies in all raccoons which showed neurological signs. In subsequent years, samples were only submitted when human exposure had occurred to saliva or other potentially infectious tissues. Swabs and tissues were collected for culture and identification of bacteria. Urine was collected and examined for *Leptospira spp.* Liver, kidney, and fat were collected from each raccoon and frozen for toxicological testing. Sera were collected, when possible, and sent for serologic testing as sample volume allowed. Diagnostic results were communicated to IDOC personnel and other designated individuals by written reports. Immediate telephone response was also given where

appropriate to the situation or requested. Data were compiled and summary tables generated to provided information on individual disease and parasite occurrence and - population-level distributions. Common syndromes causing raccoon mortality were identified.

Information on general infection rates in raccoon populations were collected from healthy raccoons cooperatively with IDNR project W-104-R-4-6, Illinois Raccoon Investigations. Data collected from sentinel raccoons included, where appropriate, serologic (distemper, leptospirosis, toxoplasmosis, parvovirus, Lyme disease), parasitologic (ectoparasites, endoparasites), microbiological and other pertinent tests. For sentinel raccoons from western Illinois, prevalence rates were calculated and risk factors for infection were determined.

Results:

During the project, 232 raccoons were necropsied. In addition, 28 raccoon tissue samples from outside sources were examined. For each animal submitted, gross post mortem findings were forwarded to the submitter by telephone or facsimile within 24-48 hours, histopathologic findings and action recommendations within 7 working days, and other test data when available.

A total of 123 non-diseased animals were necropsied and included in a Healthy Sentinel Raccoon Database. They were from Brown, Cass, Champaign, DeKalb, DeWitt, Kankakee, Union, and Will counties. A total of 109 diseased raccoons were received and examined from the following Illinois counties: Champaign, Clark, Clay, Clinton, Coles, Effingham, Ford, Jasper, Lake, Livingston, Macon, Madison, Marion, McLean, Tazewell, Vermilion, and Will counties. The 28 tissue samples were submitted from Clinton, Cook, DuPage, Jefferson, Kane, Lake, and Marion counties.

The most frequently diagnosed cause of illness or death was distemper (Table 1). Other organisms with zoonotic or cross-species disease potential found in diseased raccoons included: *Baylisascaris procyonis, Salmonella spp., Sarcocystis spp., Toxoplasma gondii, Leptospira spp., Strongyloides sp., Ancylostomatidae* (hookworms), parvo virus, and corona virus. Seropositivity rates among diseased raccoons tested were 72% (28/39) for canine distemper virus, 57% (17/30) for *Leptospira sp.* (all to serovar *grippotyphosa*, except one to serovar *icterohemorrhagiae*), 29% (6/21) for *Toxoplasma gondii*, and 91% (10/11) for parvovirus.

In the sentinel raccoon populations, seroprevalence to canine distemper virus ranged from 18% to 28% across the 3 years of the study. Distemper mortality occurred every year and one area had an outbreak in the summer of 1992. Juveniles, yearlings and adults were seropositive at each sampling period, however, adults had higher rates than younger animals. Adults born prior to 1988 had higher prevalence rates than younger animals.

Seroprevalence to *Leptospira sp.* was 48%, overall, among sentinel animals. Prevalence was lowest in juveniles (33%), but similar among yearlings (53%) and adults (58%). There was temporal and geographical variation in seroprevalence rates.

Seroprevalence to *Toxoplasma gondii* was 49% in sentinel populations. There was an increasing gradient of prevalence with age, from juveniles (14%) to yearlings (53%) to

adults (73%). Seropositivity rates were three times higher in the spring than the fall sampling periods.

All raccoons in the sentinel populations tested positive for exposure to parvovirus while no raccoons tested positive for Lyme disease.

Based on analysis of fecal samples from raccoons in sentinel populations, 38% were actively shedding eggs of *Baylisascaris procyonis*. Shedding rates were highest in juveniles (70%) and similar in yearlings (31%) and adults (28%). Eggs of *Strongyloides sp.* were shed by 15% of raccoons. Shedding rates were lower in juveniles (4%) than in yearlings (18%) and adults (19%). Shedding rates were higher in the spring (27%) than in the fall (5%). A total of 48% of raccoons were shedding lungworm (*Capillaria sp.*) eggs at the time they were sampled. Shedding rates did not differ between age groups but were higher in the spring (59%) than in the fall (40%). Raccoons were also found to be shedding eggs of the following species of internal parasites: *Eimeria sp.* (57%), *Physaloptera sp.* (1%), *Monezia sp.* (<1%), and *Ancylostoma sp.* (<1%).

Discussion:

All three objectives were accomplished during this study. Immediate response was provided to the Illinois Department of Natural Resources and the public on animals and samples presented for analysis.

The leading cause of disease among necropsied raccoons was distemper. Animals presented as both individual sporadic cases and as part of larger, geographically localized epidemics. Clinical signs observed in these animals were predominantly neurological and/or respiratory. No animals showing neurological signs, or any other signs, were found to be test positive for rabies virus. The large rabies epidemic in raccoons in the mid-Atlantic states (Krebs et al., 1993) has led to public and governmental concern whenever raccoons are seen with neurological signs. Diagnostic findings from Illinois raccoons allowed management of these cases and outbreaks as distemper alone without the potential zoonotic threat of rabies. Among sentinel raccoons, distemper also occurred both sporadically and epidemically. The presence of seropositive animals among all age groups each year showed that low-level endemic transmission was continuous, rather than only periodic epidemics. The higher levels of antibodies in adults born prior to 1988 and the observed outbreak in 1992 support the picture of an approximately four year interval between outbreaks in particular areas, as has been reported in other studies (Hoff et al., 1974; Roscoe, 1993). This may be due to a gradual increase in the total number of susceptibles over time or variation in virulence of strains of distemper virus. Distemper does not pose a hazard for humans, however, these findings highlight the need for vaccination and control of exposure potential in domestic canines. These findings also have implications for management of free-ranging raccoons in Illinois zoos and animal preserves. Recent deaths of exotic felids have been reported in Illinois and elsewhere, due to canine distemper virus believed to have been of raccoon origin (Appel et al., 1994). Further, translocation of raccoons may provide a means for introduction of distemper virus into new areas. Even apparently healthy raccoons should not have contact with susceptible animals of their own or other species, if spread of distemper is of concern.

The histopathologic examination of raccoon tissues in this study documented the lesions

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caused by distemper virus and other disease agents in the brain, lung, and other organs of affected animals. Of particular importance, is the observation that distemper in raccoons can produce encephalitis, proliferative pneumonia and lympholysis. The proliferative pneumonia has been reported previously in isolated cases of raccoon distemper but this study confirmed the importance of this lesion as a contributing factor in raccoon death losses associated with distemper infection. Equally important is the observation that many raccoons infected with distemper virus have concurrent brain and probably lung infections with a protozoal-like agent resembling *Toxoplasma spp./Sarcocystis spp.* These agents are often primary infectious agents in diseases processes but more commonly are active in animals that have their immune systems suppressed. A final important observation in the histopathologic study was the finding of destruction and loss of lymphoid tissue in lymph nodes. The combination of lymphoid loss and secondary infections with *Toxoplasma spp./Sarcocystis spp.* suggest that distemper virus causes generalized suppression of the raccoon's immune system resulting in susceptibility to other zoonotic parasites and diseases. Raccoons that recover from distemper may acquire other diseases later in life that may result in local or regional die-offs due to some other less common disease process.

Although the lesions observed in raccoons with distemper have been described in several case reports, these observations were based on individual animals in isolated regions. The lesions in the nervous systems of distemper virus-infected animals are similar in most common domestic and wild animal species. The proliferative pneumonia observed in exotic cats (Appel et al., 1994) is similar to that seen in Illinois raccoons. Our study documented the character and severity of the lesions and confirmed that the proliferative lung lesions are not an isolated occurrence but a common and important finding in raccoon distemper. Such lesions when combined with the observation of lymphoid depletion suggest that raccoon distemper is an important immunosuppressive disease that allows other secondary agents such as bacteria or protozoal parasites to cause concurrent or delayed disease in raccoon populations. Additional studies documenting these lesions and characterizing the importance of this immunosuppressive syndrome are in progress.

The six animals diagnosed at necropsy with clinical leptospirosis, and seroprevalence rates of 57% among diseased animals and 48% among healthy, sentinel animals suggest that this pathogen is wide-spread among raccoons. Animals may die with acute illness, or long-term infection can lead to pathologic changes in kidneys and liver of older animals (Shotts, 1981). In several animals, clinical leptospirosis seemed to be a sequelae to immunosuppressive effects of previous canine distemper infection. Both serovars identified in these raccoons (*grippotyphosa* and *icterohemorrhagiae*) can be spread to humans and domestic animals, causing serious disease. Serovar *grippotyphosa* was the cause of an outbreak in teenage boys in Illinois associated with swimming in a woodland pond (Jackson et al., 1993). Infective urine is the primary source for transmission, either through direct contact, through feed contamination or through water. The organisms can survive for several weeks in warm, alkaline soil, stagnant ponds or slow-moving, alkaline streams (Shotts, 1981). Recognition of the ubiquity of this pathogen in raccoons and the probable environmental contamination of areas they frequent is important in decreasing human and domestic animal exposure. People handling raccoons should wear gloves and those engaging in recreational activities in areas of high raccoon density should be made

aware of the risks. Hawaii has signs posted along streams and wetlands, warning of the hazards of leptospirosis, however, the risk there is higher because of the warmer climate.

The definitive hosts for *Toxoplasma gondii* are cats and feline species are the only type able to shed the eggs (oocysts). When oocysts are ingested by an animal, they migrate to muscle tissue and encyst. Infections in humans and other species occur from exposure to oocytes shed by cats or through consumption of meat containing encysted *Toxoplasma* organisms. The presence of *Toxoplasma* in raccoons therefore serves as a sentinel measure of environmental exposure to *Toxoplasma* as well as a source of infection for humans who consume raccoon meat. The high seroprevalence in sentinel raccoons and the increase with age (14% in juveniles to 53% in yearlings to 73% in adults) also suggest toxoplasmosis to be a common infection in raccoons. Although *Toxoplasma* infection does not generally cause disease in raccoons (Dubey et al., 1992), a number of raccoons which tested positive for canine distemper virus were found to have lesions in the brain associated with *Toxoplasma*-like agents. The high mortality and frequent neurological signs, seen in some areas with distemper in raccoons, may be related to activation of *Toxoplasma* or a related organism following immunosuppression by the distemper virus. This has been hypothesized in several cases from other parts of the country (Dubey et al., 1992). Further work to characterize and report the lesions and responsible agent are in progress.

Almost all the raccoons tested were positive for parvovirus infection, both in the sentinel population and among diseased raccoons, although only nine cases of clinical disease were diagnosed. This virus is closely related to the virus causing feline panleukopenia. Previous reports of disease in raccoons due to this virus have been in animals in captivity (Nettles et al., 1980). The importance among wild populations is not known. The finding in this study of clinical cases among free-ranging animals suggests that this disease does effect feral populations. Of the nine clinical cases, six were also affected with distemper. Two were diagnosed from submitted biopsy samples, so that co-infection with distemper could not be evaluated, and one was a juvenile raccoon from an outbreak of diarrhea at a rehabilitation facility. This suggests that parvovirus infection may be primarily a problem in raccoons if another stressor is simultaneously present. This infection is not transmissible to humans, but does pose a hazard for cats and other wild carnivores. Publication of the findings on clinical cases of parvovirus disease in free-ranging raccoons is in progress.

Although none of the intestinal parasites identified among the diseased or sentinel raccoons were associated with health problems in these raccoons, a number of these agents are zoonotic. The high shedding rates of *Baylisascaris procyonis* eggs from healthy raccoons (70% in juveniles and ~30% in yearlings and adults) and the high prevalence of these roundworms in diseased raccoons (45%; 42/94) is similar to results from other studies (Kazacos and Boyce, 1989). Larval stages of this parasite can cause visceral, ocular and cerebral larval migrans in humans and more than 40 species of mammals in North America (Kazacos and Boyce, 1989). At least two human deaths have occurred due to larval migrans (Cunningham et al., 1994). Based on the pervasive presence of this parasite in Illinois raccoons, measures should be taken to prevent contact between susceptible species, especially humans, and raccoon feces or contaminated ground. Raccoon feces should not be allowed to accumulate in areas used recreationally by

humans or around homes. Facilities to permit handwashing should be readily accessible in picnic areas. The public should be informed that raccoon worms can pose a health hazard and that good sanitation is essential to prevent infection.

Human exposure to larval stages of *Strongyloides sp.* (present in 15% of healthy raccoons and identified in only 3 diseased raccoons) and *Ancylostomatidae* (hookworm) species (identified in 56% of diseased raccoons) can lead to skin lesions as larval forms migrate through the skin. Although these are usually mild, self-limiting and occur only in areas of skin in contact with contaminated soil, they can be irritating while present. The frequent identification of these species from raccoons provides further justification for preventing human contact with soil contaminated by raccoon feces and for providing facilities for washing skin in recreational areas.

Other internal parasites detected in Illinois raccoons pose limited or no zoonotic potential, and hygienic measures which prevent exposure to feces and soil contaminated with feces will also ameliorate risks due to these parasites. Although this study did not examine prevalence of *Giardia* or *Cryptosporidiosis*, good sanitation would also provide a barrier to infection with these agents.

Table 1
Summary of Diagnostic Findings for Raccoons, 1992-1995

Diagnosis	1992-1993	1993-1994	1994-1995	Total
Distemper	35	53	18	106
Protozoal encephalitis	4	2	4	10
Parvo virus infection	3	4	2	9
Corona virus infection	2	2		4
Sarcocystosis	2	11	6	19
Leptospirosis	2	2	2	6
Salmonellosis	1	2		3
Bacterial dermatitis	1			1
Bacterial septicemia	1			1
Fungal encephalitis		1		1
Lungworm infection	28	26	4	58
Hookworm infection	21	22	6	49
Baylisascaris infection	15	20	14	49
Coccidia infection	23	30	3	56

Other intestinal parasites occasionally identified in raccoons: *Mesocestoides, Physaloptera, Atriotaenia, Strongyloides, Macracanthorhynchus, Trichuris, Arthrocystis, Cruzia*

Job 1.2: Initiate technical support and develop professional expertise for data collection and response.

Objectives:

1. To implement an effective staged strategy for response to changing health conditions in raccoon populations.
2. To provide immediate and long-term expertise for IDNR personnel when responding to disease problems in raccoon populations.

Materials and Methods:

To develop a plan for staged response to changing health conditions in raccoon populations, initial discussions were held with IDNR administrators, followed by discussions with selected field personnel to identify the specific needs of the department and to evaluate feasibility of sample collection and outbreak investigation methods. These were accomplished via telephone interviews. A meeting was held in conjunction with the first training session for field personnel to discuss and further develop the plan for staged response. After development of the plan, it was applied for the subsequent two and a half years.

Results and Discussion:

Procedures for telephone consultation, sample submission and initiation of raccoon disease outbreak investigation were developed. A plan for staged response to possible raccoon health problems was generated and implemented. Three levels of technical support and expertise were outlined to respond to wildlife health problems. For more routine problems, IDNR personnel received additional, specialized training to enhance their ability to make field evaluations and to select, prepare and submit samples as needed. This training was provided through two annual full-day training sessions, a field manual, reference books and materials, a field necropsy kit and a computer tutorial program for individual home-study, installed on a Power Book 165C Color portable unit (see Job 1.3). Back-up support for stage 1 was provided by Drs. Zachary and Hungerford via telephone and facsimile, as needed. Initially this consultation was limited to IDNR personnel, however it was expanded in the second year to include members of the general public referred by IDNR personnel. The protocol for submission of samples for rabies diagnosis was also modified slightly so that heads and/or brains could be submitted directly to the Centralia Diagnostic Laboratory, and a portion subsequently forwarded to University of Illinois for other testing. At the second level, in the event of a major raccoon problem, a list of available experts, with a range of expertise, was assembled from the University of Illinois, College of Veterinary Medicine. These personnel would be available to travel to the site to assist IDNR personnel with the problem. The third level of support emphasized more basic training and continuing education for IDNR field personnel, to prepare them to manage a wider range of disease situations and decrease future need for higher level support. Effectiveness of the three stage response was evaluated in conjunction with the second training seminar in 1994 (see Job 1.3).

IDNR personnel were able to resolve most raccoon health problems during the project

with support provided at the Stage I level. Drs. Hungerford and Zachary consulted with IDNR personnel to provide additional information and recommendations. Issues addressed included: a variety of questions on zoonotic diseases of raccoons, coyotes and deer, distemper disease outbreaks in Cook, Champaign, Lake and other Illinois counties, appropriate techniques, drugs and equipment for capturing and euthanizing raccoons, sample submission for various species and types of special tests, tularemia in deer, distemper in foxes, chemical immobilization and reversal techniques for a number of wildlife species, radio collaring raccoons, fungal diseases in deer (with respect to a mass herd die off in a corn field), vaccinations for farm raised raccoons, the zoonotic potential of raccoon distemper, differential diagnoses for different disease syndromes in raccoons, diseases in squirrels, death of a swan in Kankakee county, diseases of coyotes, problems dealing with wildlife native to Illinois, food safety concerning euthanasia solution, clean up of a barn contaminated by raccoons, the interaction of raccoons, dogs and human zoonoses, parvovirus in raccoons, potential zoonotic causes of skin disease in a human in contact with wildlife, zoonotic potential of exposures to house mice, diseases of squirrels, differentiation of distemper and rabies in raccoons, ehrlichiosis in Illinois wildlife, methods used to combat the spread of rabies among east-coast raccoons, shedding of antlers by white-tailed deer, ticks and Lyme disease in Illinois and zoonotic potential for exposure to baby wild birds in a daycare setting.

A press release was prepared and a newspaper article was published regarding investigation of several regional distemper outbreaks. Dr. Hungerford also presented a talk to the public on zoonoses of raccoons and she and Dr. Zachary presented project findings and general information to the annual IDNR meeting.

Job 1.3: Development and implementation of basic and continuing education workshops and materials for IDNR personnel.

Objectives:

1. To teach IDNR personnel the techniques to select and prepare tissue and other samples for submission to the diagnostic laboratory for evaluation.
2. To design and develop computerized instructional materials to assist IDNR personnel in managing disease outbreaks in raccoon populations.

Materials and Methods:

To effectively implement the staged response, a program was developed to enhance the expertise of IDNR personnel with regard to disease problems in raccoon populations. Two primary types of instruction were used: workshops and computer-aided learning. Two full day workshops were developed for IDNR personnel. Topics were selected and teaching materials prepared to provide background information, hands-on skills and opportunities for discussion and sharing of the knowledge-base already present within the group. One of the pathologists (Dr. Grant Rezabeck) from the State Laboratory of Diagnostic Animal Medicine at Centralia was involved in the second seminar to help integrate the project across the state. Each seminar used a

series of lectures, discussions and laboratory sessions. Handouts, a field reference manual, a field necropsy kit and selected reference materials were selected and distributed to participants.

A computer tutorial, developed by Dr. J. Zachary with help from Dr. L. Hungerford and the veterinary student assistants, was selected as the method to accomplish the computer-aided instruction. The tutorial was designed to allow IDNR personnel to independently study diseases and diagnostic methods for raccoons at their work sites. It was structured to allow the user to determine his/her own path through the presented material and to easily review and compare sections. The basic structure of the computer software program for the tutorial was designed first and then digitized images were assembled, indexed and edited. Text was integrated with the digitized images of raccoon necropsies and diseases. The completed program was installed on a Power Book 165C Color portable unit and beta tested by the co-investigators and then by selected IDNR personnel. After refining the program based on beta test results, the unit and software were made available via the mail for IDNR personnel to share and use for training in their job sites.

Results and Discussion:

Two full day workshops were held on Field Methods for Evaluating Raccoon Health. Each was attended by approximately 20 IDNR personnel. Each seminar used a series of lectures and discussions to focus on epidemiologic evaluation of diseases, common infectious diseases, selection of samples for investigating disease outbreaks, mechanisms of disease transmission, and interpretation of post-mortem lesions and artifacts. Handouts on transmission of infectious diseases, investigation of disease outbreaks, and zoonotic diseases of raccoons were provided. Each participant also received an overview of the project and sample submission forms. A field reference manual which provided readily accessible information that reinforced the materials presented in the seminar was also developed as part of the project and provided for the participants (Appendix A). At the second seminar, each participant also received a field necropsy kit complete with necessary equipment for necropsy and tissue collection and a copy of *Zoonoses and Communicable Diseases Common to Man and Animals* (Acha and Szyfres, 1987). During the first workshop, a laboratory session was held so that each participant could perform a post-mortem examination on a raccoon under the guidance of College of Veterinary Medicine faculty. The proper techniques for selecting and preparing tissue and other samples for submission to the diagnostic laboratory demonstrated and given during this lab. At the second workshop, a slide presentation on diseases carried by wildlife with an emphasis on zoonotic diseases of raccoons was presented by Dr. Hungerford. Dr. Grant Rezabeck from the Laboratory of Diagnostic Animal Medicine at Centralia demonstrated use of the field necropsy kits to prepare tissue samples for collection and mailing for histopathological analysis.

Drs. Hungerford and Zachary provided additional continuing education at the Illinois Department of Conservation and Department of Wildlife Resources Annual Meeting in Mount Vernon on March 9, 1993. They presented information on the types of diseases and parasites found in raccoons during the project and on zoonotic diseases important to conservation personnel.

Color slides were taken of necropsy procedures for use in the seminars and for incorporation as illustrations in the field reference manual. These were also scanned into the computer for use as digital images in tutorial programs. Histopathologic slide samples were photographed and scanned into the computer to use in the tutorial program.

A computer tutorial was developed to provide independent self-review and instruction for IDNR personnel (screen prints included as Appendix B). The tutorial covered gross lesions and diagnostic methods for raccoons. The completed program was installed on a Power Book 165C Color portable unit. This portable unit was selected so that the tutorial could be made available via the mail for IDNR personnel to share and use for training in their job sites. The portable computer was used by IDNR personnel during the project to study and to send messages to each other and to Drs. Hungerford and Zachary. This now serves as a continuing tool for independent training and problem-based learning about techniques for field diagnosis of diseases in raccoons.

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FIELD MANUAL FOR THE RACCOON (*PROCYON LOTOR L.*).

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I. INTRODUCTION

1. Need:

Recent declines in harvest pressure and pelt prices have lead to a marked rise in raccoon numbers in Illinois. The resulting high populations and increased animal densities have created conditions which facilitate spread of parasites and disease between animals and can lead to disease epidemics. Many parasites and diseases of wild animals also can affect humans and domestic animals. Raccoons serve as major carriers and definitive hosts for a variety of important diseases of humans and animals including rabies, leptospirosis, roundworms and canine distemper (Acha and Szyfres, 1987; Kaufmann, 1982). Illinois raccoons do indeed carry these agents, but at present there are no data to determine if and when such cross-transmissions occur in Illinois. In addition, outbreaks of non-zoonotic diseases in raccoons cause alarm in individuals near or exposed to these diseased raccoons. Since these changes in wild populations directly impact both human and animal health, surveillance is necessary for both wildlife management and public health concerns. The IDOC must take appropriate action under these circumstances to protect wildlife, respond to public concerns, and resolve potential zoonotic disease outbreaks in a timely and accurate manner.

Currently no organized and focused mechanism exists to rapidly identify, diagnose, evaluate, and resolve potential or existing zoonotic disease problems in urban or rural raccoon populations within Illinois. Ongoing parasite and disease surveys also are needed to monitor and predict disease problems and to permit evaluation of disease interactions with harvest programs. The establishment of a cooperative program, which links and enhances the expertise of IDOC/DOWR personnel with the resources and expertise of selected faculty at the University of Illinois College of Veterinary Medicine (UICVM), will develop the necessary resources to be used for making health decisions and will provide an effective pattern for response in these situations. Interactive laboratory (computer) and field (workshop) experiences are needed for IDOC/DOWR personnel to gain experience and confidence in participating in this research project. This training approach will be facilitated with computer simulated outbreaks of raccoon diseases and computer auto-tutorials that focus on post-mortem techniques, lesion evaluation, and sample preparation and submission.

2. Objective:

The general objective of this project is to develop a cooperative interagency strategy for diagnosing, monitoring, managing, and evaluating infectious and parasitic zoonotic diseases of raccoons that could have deleterious effects on citizens as well as wild, companion, and food-producing animals of Illinois.

3. Expected results and benefits:

This project will build closer collaborative ties between IDOC/DOWR personnel and the UICVM in addressing the mutual goals of improving animal and public health. The

cooperative program will monitor disease trends in sentinel, sick, and dead raccoons in Illinois and develop data to allow assessment of zoonotic hazards known to be present in Illinois. These data will provide IDOC/DOWR personnel, appropriate state agencies, and public health officials with immediate information about sudden and potentially zoonotic disease outbreaks as well as long term data that will relate raccoon disease trends (health status) with population trends.

IDOC/DOWR personnel also will acquire basic instruction and continuing education on field diagnostics and management of endemic and epidemic zoonotic diseases to strengthen combined University/State expertise. This will allow timely and appropriate response to and management of public health problems that arise from outbreaks of disease in raccoons with zoonotic potential, such as rabies, and furnish cumulative population health data that can be used to make reasonable judgements in long-term management of raccoon populations.

II. GENERAL SUGGESTIONS

1. Description of Abnormalities

Every effort should be made to describe the abnormality as accurately as possible. Formalin will change the color and texture of the lesions. Include on the necropsy form the color, size, shape, and texture of the lesion.

2. Abnormal Tissue Collection

The abnormal tissue collected should have normal tissue with it as part of its submission. Often, the morphologic changes associated with abnormal tissue make it impossible to identify the tissue of origin. This fact makes it important to include some normal tissue on the periphery of the lesion as part of its submission.

3. Precautionary Steps

Raccoons are known carriers of diseases which can be transferred to humans and other animals. With this in mind, it is advisable to avoid touching the raccoon's body directly. Rubber gloves and an apron should be worn at all times when handling the raccoon's body. When finished with the necropsy procedure, thoroughly disinfect yourself and any clothing,including shoes that may have become contaminated during the necropsy. These precautionary steps will help reduce the risk of exposure to contaminants by the person doing the necropsy and any animals that person will subsequently come in contact with.

4. Labels

Labels are as important as the sample itself. Each sample should have the field number and type of sample on its label in waterproof ink. Completion of the necropsy includes ascertaining that each sample has a label and is securely stored for transport. Label all suspect zoonotic disease specimens (such as rabies, brucellosis, etc.) conspicuously on the outside wrapper as well as on the individual specimen container.

III. SUPPLIES

1. General Supplies

   a. Fixatives - 10% neutral buffered formalin, 70% ethanol

   b. Labeling supplies

   c. Sample containers

   d. String for tying off intestinal loops

   e. Pencils

   f. Necropsy forms

2. Necropsy Tools

   a. Scalpel blades and handles

   b. Small and large dissection scissors

   c. Long and short handled tweezers

   d. Large, sharp skinning knife

   e. Wetstone

   f. Wire cutters for cutting ribs - (should be able to clean them easily)

   g. Protective clothing - rubber gloves, aprons, etc.

   h. Tape measure

   i. Portable scale - (spring or platform scale)

   j. Clipboard

   k. Dissecting tray or board

   l. Submission forms

IV. DELIVERY, PACKAGING, AND MAILING INSTRUCTIONS

1. Intact Carcass

Necropsy (post-mortem examination) must be performed as soon as possible after death because tissue autolysis and putrefaction begin immediately. The chances of demonstrating or recovering disease-producing microorganisms and of recognizing significant tissue alterations diminish rapidly as post-mortem changes advance. Refrigeration of a carcass is helpful in slowing the advance of such changes, but it must be remembered that it requires many hours for the internal organs to reach an inhibitory temperature (~40°F). The time required for this depends on the size of the animal, amount of fat, and thickness of the hair coat. In large animals, it may take 48 hours or more. If refrigeration is not possible, a carcass should be doused with cold water immediately after death and placed in the coolest area possible until necropsy can be performed.

Carcasses may be frozen if a long delay (several days or more) before necropsy is anticipated. However, freezing causes the formation of ice crystals in the tissues which often precludes satisfactory interpretation of disease-induced tissue changes. It also significantly reduces the likelihood that bacterial and fungal agents in diseased tissues can be successfully isolated and identified. On the other hand, viral agents and many toxic substances are better preserved by freezing than refrigeration.

2. Packaging, Preservation, and Shipment of Animal Specimens

Specimens of various types which are to be shipped or mailed to the laboratory for examination must be properly packaged and preserved for temporary storage and shipment. See information and instructions for specific laboratory sections when mailing specimens for examination.

When preserved specimens are sent by parcel post, enclose the clinical history and billing information in a stamped envelope and attach it securely to the exterior of the package. Written material may be placed inside the package if it is to be shipped by express mail. However, the letter must be inside plastic, protected from contamination by fluids from the specimen or destruction by condensation from chilling devices.

Specimens should be mailed so they do not arrive on a weekend and/or holiday. Preserve the specimens until they can be sent for arrival on a working day. In most cases, this will involve refrigeration, but if in doubt, check with the appropriate laboratory.

Make sure all packaged specimens are clearly labeled with appropriate identification designating them as part of this raccoon study with the state of Illinois. Labels should also include the field number and the nature of the specimen (liver, serum, stomach content, etc.). For the protection of laboratory personnel, label all suspect zoonotic disease specimens (such as rabies, brucellosis, etc.) conspicuously on the outside wrapper as well as on the individual specimen container.

V. PRE-NECROPSY EVALUATION

The animal's history will aide the diagnostician's evaluation of the animal. Record on the necropsy form the exact location where the animal was found, including county, city, and street. In the history section of the necropsy form record the sex of the animal, the condition of the animal when found (fresh, fairly autolyzed, severely autolyzed), and whether it is alive or dead. If dead, also record the length, weight, estimated age, and nutritional condition. These measures are taken after euthanasia in live animals.

Before the necropsy begins, a thorough evaluation of symptoms and external bodily conditions should be recorded (see form in appendix E). These categorizations will help you select the proper necropsy technique for your animal.

If the animal is alive, its behavior should be noted and categorized. Central nervous system (CNS) involvement is indicated by abnormal behavior. Abnormal behavior can be described as circling endlessly, blindness, staggering, self mutilation, a stiffly gaited walk and/or daytime activity. Animals in a stuporous condition or exhibiting rapid, foamy jaw movement can also be considered to have CNS involvement. This should be noted on your necropsy form. For the animal that is presented dead, mark as "Don't know".

Once you've established whether or not the animal has CNS involvement, you should examine its bodily condition, (dead or alive). It is necessary to determine whether the gastrointestinal (GI) tract is involved. This should be performed by simple visual examination and the animal should not yet be handled. Visual examination of the anal area for diarrhea staining of fur or scabbing/bleeding of the anus indicates GI involvement. Hair loss around the anal area and on the caudal margins of the legs also may be considered positive indications. If the animal has diarrhea it should be noted on the necropsy form. An animal that appears bloated, has a swollen abdomen, or emaciation should be considered in this category.

Symptoms of respiratory disease include discharge from the nose and eyes, coughing, wheezing, sneezing, etc. These symptoms are noted under "Respiratory signs" on the necropsy form. Again, dead animals must be marked as "Don't know".

The "Comments" section of the form is for the description of any abnormal or unusual external findings not included as a category on the form. Examples include evidence of hair loss or poor coat condition, half-eaten animals, malformations, severe dental problems, etc. This is to note conditions that may be the cause or result of the animal's general state of health.

VI. POST-MORTEM EVALUATION

1. Interpreting Color Changes in Tissues

Color	Interpretation of tissue or organ change
Red or Dark red-purple or Red black	sudden blood loss with tissue destruction sudden congestion
Red-yellow or Brown	older blood loss with tissue repair older congestion
Brown-yellow or Brown	oldest blood loss with tissue repair oldest congestion
Yellow-orange	bile pigment staining of abdominal organs bile pigment staining of all tissues accumulation of fat in an organ dietary intake of carotene fat (myelin) breakdown
White	fibrous repair following injury cellular degeneration in tissue or organ accumulation of cells in tissue or organ accumulation of water in cells anemia if mucous membranes
Black	melanin pigment pseudomelanosis (post-mortem putrefaction)
Green	bile pigment bacterial pigment
Blue	oldest blood loss with tissue repair toxins in blood stream (mucous membranes)

2. Describing Color Changes and Lesions

a. Location: Name the area of body, organ, or tissue affected

b. Distribution: focal, multifocal, diffuse, interstitial, locally extensive, embolic, lobar, etc.

c. Color: red, red-brown, brown, pale red, etc.

d. Shape: round, nodular, irregular, etc.

e. Surface Conformation: raised, level, depressed, umbilicated.

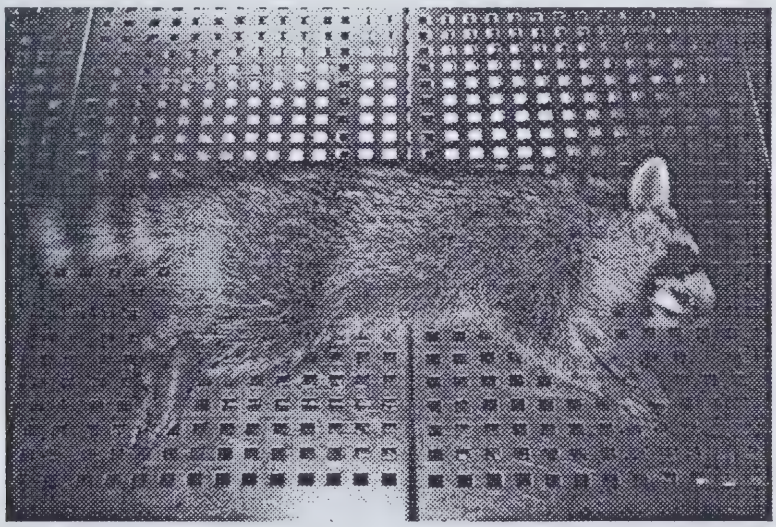
f. Size: dimensions in metric units.

3. Post-mortem Evaluation (This technique is for an in-house necropsy. Performing necropsies in the field may abbreviate or change some of these procedures when necessary)

a. Check or assign animal field number
   (1). Review history.
   (2). Review protocol. (NOTE: Accession number will be assigned by the diagnostic lab.)

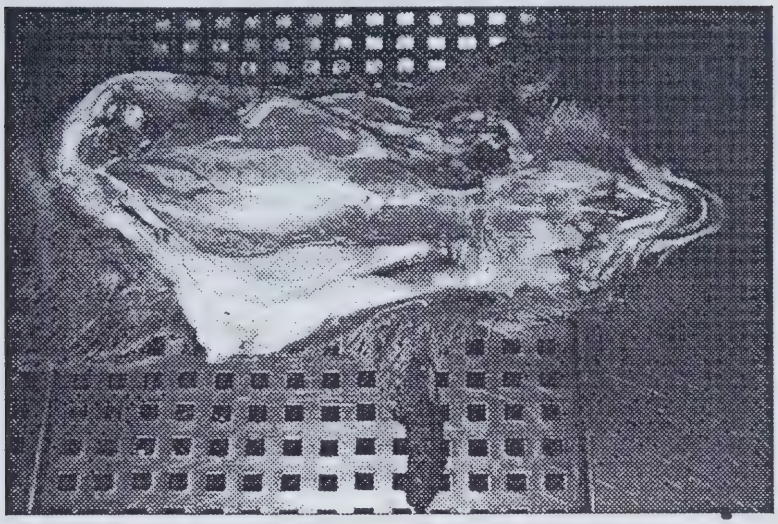
b. Method of euthanasia
   (1). First, a preanesthetic injection of Ketamine-Xylazine is given.
   (2). Induce asphyxiation in a carbon dioxide chamber.
   (3). Asphyxiation is followed by a bleed out through the femoral arteries.
   (4). Collect at least 6cc of blood from the bleed out site.

c. External examination



   (1). Examine carefully the entire body surface, orifices and mucous membranes. Record the condition of teeth if they appear abnormal.
   (2). Note condition of skin, hair-coat, and body in the "Comments" section.
   (3). Collect and preserve ectoparasites if present; note location of ectoparasites.

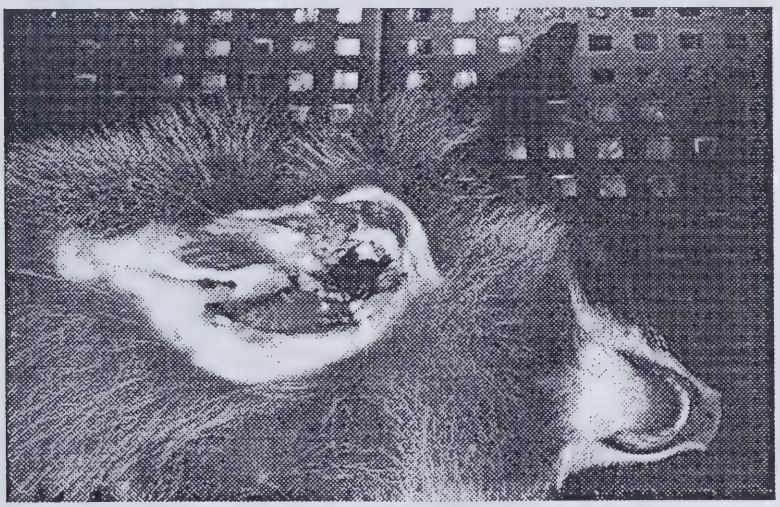
(4). After the external examination is complete,



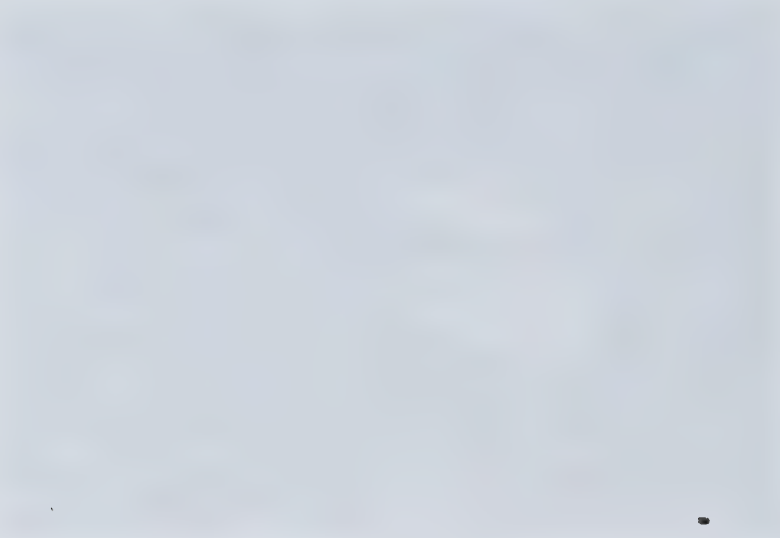
incise the skin along the right ventral side of the right forelimb and hindlimb, starting the incision at the nasal bone and continuing to the sacrum and reflect to the left.

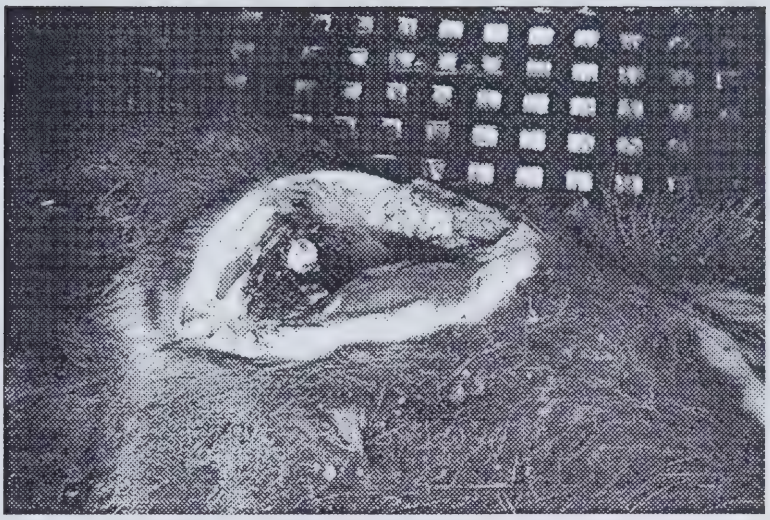
(5). Examine for any hemorrhage or bruising in or beneath the skin and muscular areas. Trauma to the CNS will be manifested in these areas; omitting this step at the beginning will complicate evaluation by the migration of blood into the subcutis from ventral incisions. Note any findings in the "evidence of trauma" section.

d. Collection of lymph nodes (two sites)



(1). Pectoral limbs are reflected by armpit incisions, and axillary nodes are exposed with this incision and collected.

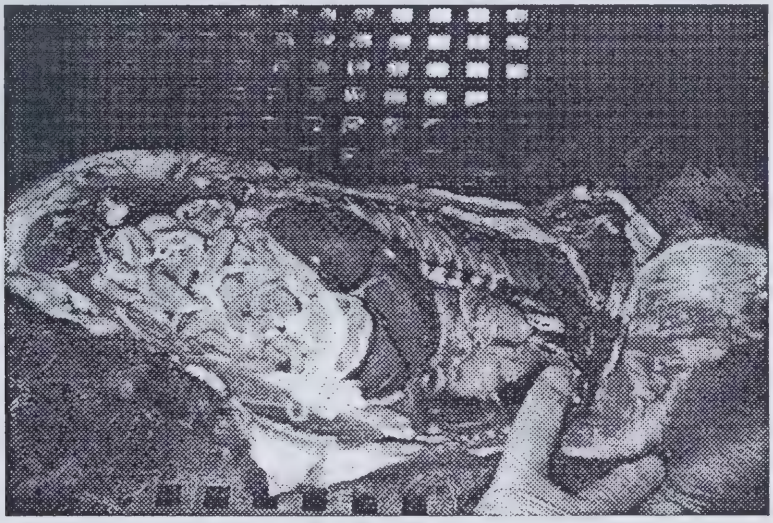




(2). Pelvic limbs are reflected by incisions through the groin muscles. Continue to the hip joint with incision of the joint capsule, and the joints are then exposed for observation.

(3). Incisions along the caudal and medial aspect of the thigh (just behind the knee) will expose the popliteal lymph nodes to be collected.

e. Opening the abdominal and thoracic cavities



(1). Open the abdominal cavity by cutting along the caudal margin of the rib cage, beginning at the middle and continuing to a mid-lateral position, a few inches from the vertebral column. Now extend the incision caudally and along the medial border of the thigh to the base of the tail.

(2). Reflect the resulting abdominal wall flap medially.

(3). Examine the cavity contents *in situ*, looking for peritoneal fluid and organ displacements.

(4). Nick the diaphragm to cause the diaphragm to move caudally as air is sucked into the thoracic cavity. If the technique is used correctly, failure of the diaphragm to move indicates ante-mortem collapse of the lung and pneumothorax.

(5). Remove the sternum by incising near the costochondral junction of the left and right side of the sternum with wire cutter or shears.

(6). Examine the thoracic contents *in situ*; nick the pericardium and examine the contents of the pericardial sac.

(7). Collect a urine sample from the bladder with a sterile syringe and needle.

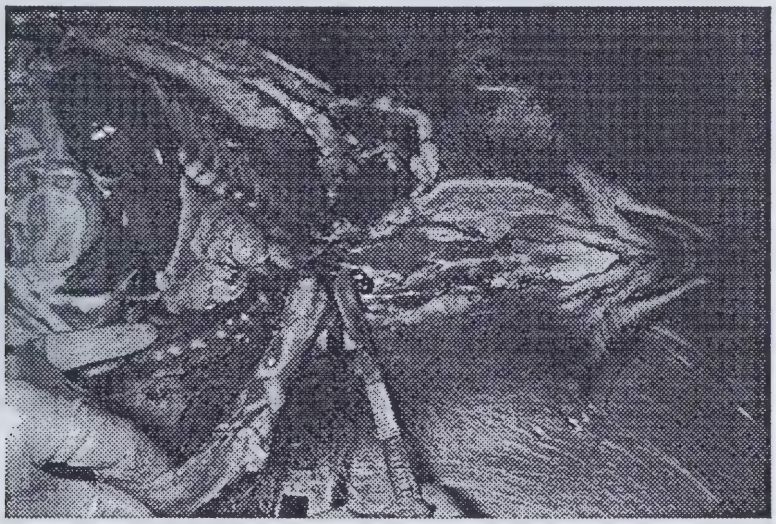
(8). If CNS or respiratory signs are present, collection of the urinary bladder is also recommended. It should be cut open and preserved in formalin.

(9). Collect samples for *Salmonella* sp culture (liver, SI, spleen). Using sterile technique place the samples into sterile petri dishes or sterile Whirl-paks.

f. Collection of the thoracic pluck

(1). This pluck includes tonsil, lungs, and heart.

(2). Carefully trim the thyroids with the parathyroids (normally these will appear as one gland) from the trachea.



(3). Incise the soft tissue on both sides of the tongue and the hyoid bones. Exert gentle traction on the tongue while cutting under and along the trachea, pulling upward as the cuts are being made, until the entire lung pluck has been freed. As the tonsil is encountered, temporarily interrupt the removal of the pluck to examine and remove the tonsils.

(4). Examine the tongue and make multiple transverse incisions, looking for focal lesions.

(5). Reflect the pericardial sac and remove the heart from the lungs by cutting under the heart, severing the arteries.

(6). Hold the heart in your left hand with the apex towards you, and with scissors make a cut in the right ventricle adjacent to the septum and extend the cut dorsally and caudally into the caudal vena cava.

(7). Extend the cut anteriorly from the posterior vena cava, opening the cranial vena cava.

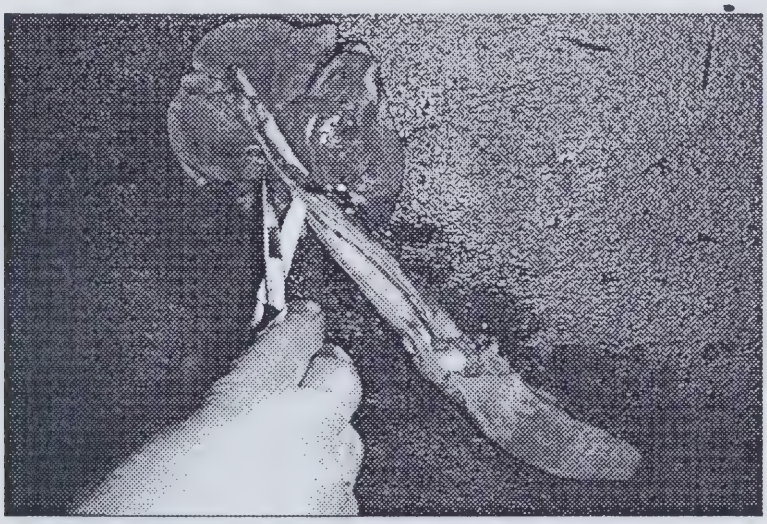
(8). Extend the initial cut (6) in the right ventricle dorsocranially adjacent to the septum, continuing out to the arteries.

(9). Examine all valves and openings of the right auricle and ventricle.

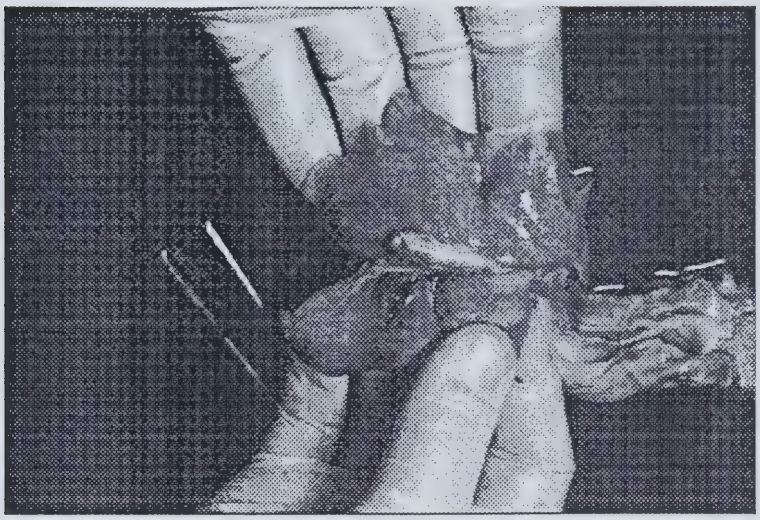
(10). Rotate the heart counter-clockwise until the left auricle is uppermost. Hold the heart in your left hand with your thumb over the left auricle, bisect the left ventricle near the apex, and extend the cut dorsally into the pulmonary veins. Examine the left AV valve.

(11). With your left index finger and thumb, grasp the left half of the ventricle. With scissors in your right hand, cut through the left ventricle, going under the left AV valve.

(12). At this time collect a tissue sample from the heart. This is best collected by placing the now opened heart into formalin.



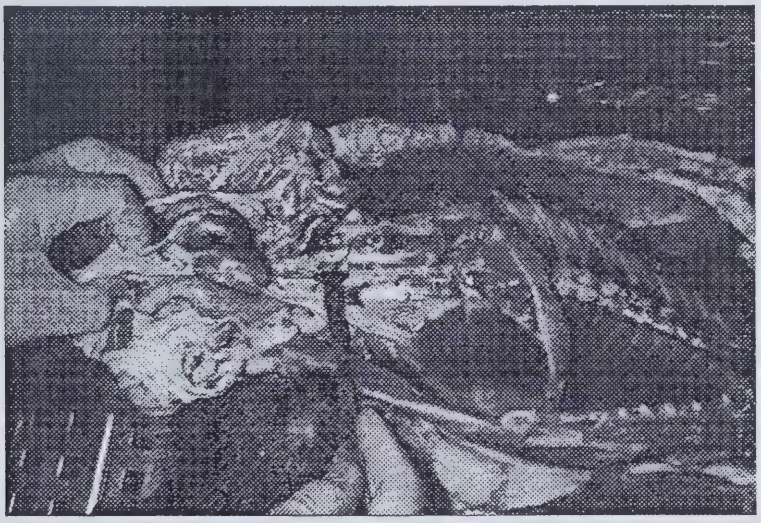
(13). Incise the wall of the esophagus longitudinally and inspect the mucosal surface. Incise the larynx and trachea longitudinally, inspecting the mucosal surface.



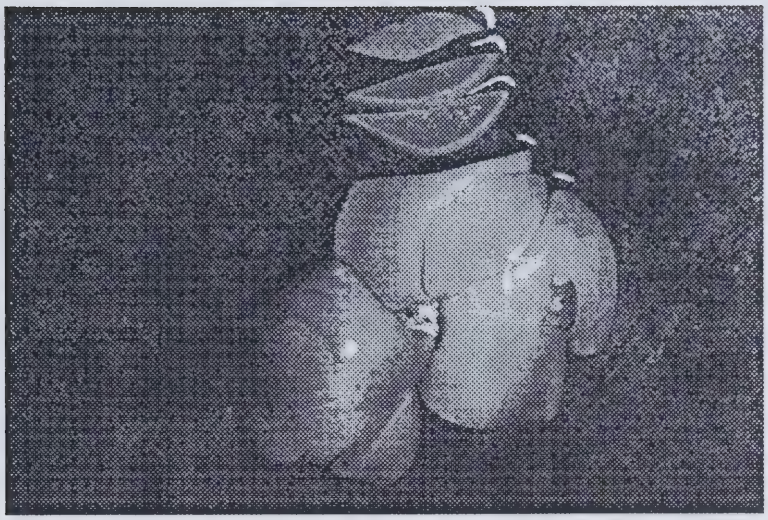
(14). Palpate and inspect the lungs. Open the tracheal-bronchial tree to the furthest extremities. In the absence of gross alterations, the lungs are adequately sampled for histology by selecting sections from the animal's left uppermost lobe and its right rear lobe.

g. Collection of abdominal pluck

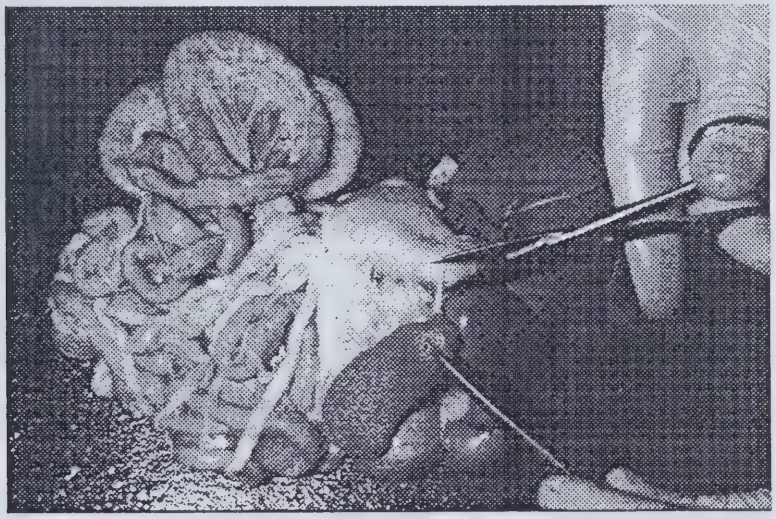
(1). This pluck includes the stomach, small and large intestine, mesenteric lymph nodes, spleen, liver with gallbladder, pancreas, adrenals, and kidney.



(2). The gastrointestinal tract, spleen, liver, kidneys, adrenals and pancreas are removed by gentle traction and incision. Starting at the kidneys, cut under the kidneys lifting as you cut.



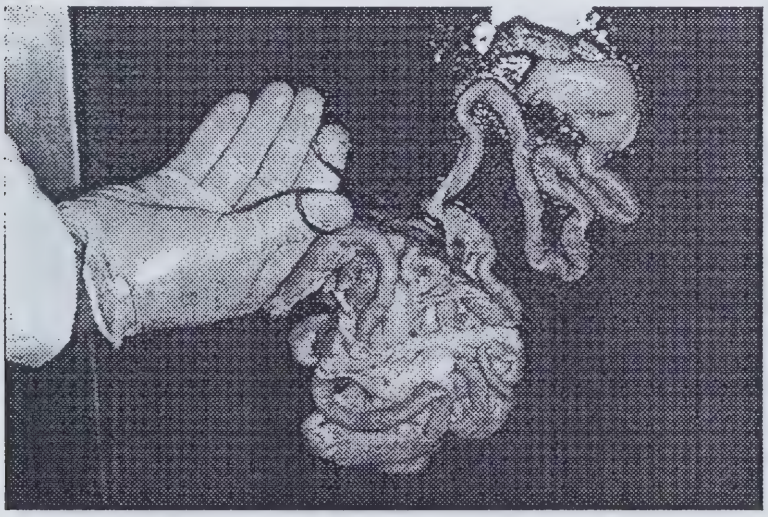
Place the liver on a cutting board for examination; make multiple incisions in the organ to avoid missing small focal lesions not extending to the surface. Sections for histology in the absence of any lesions should include a section from the left and right major lobes and a section including gallbladder.



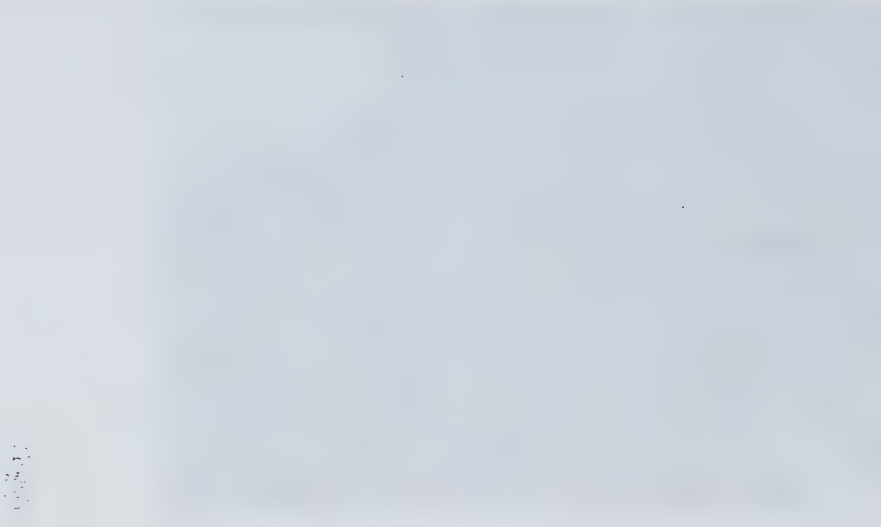
(7). Trim, inspect, and palpate the spleen.

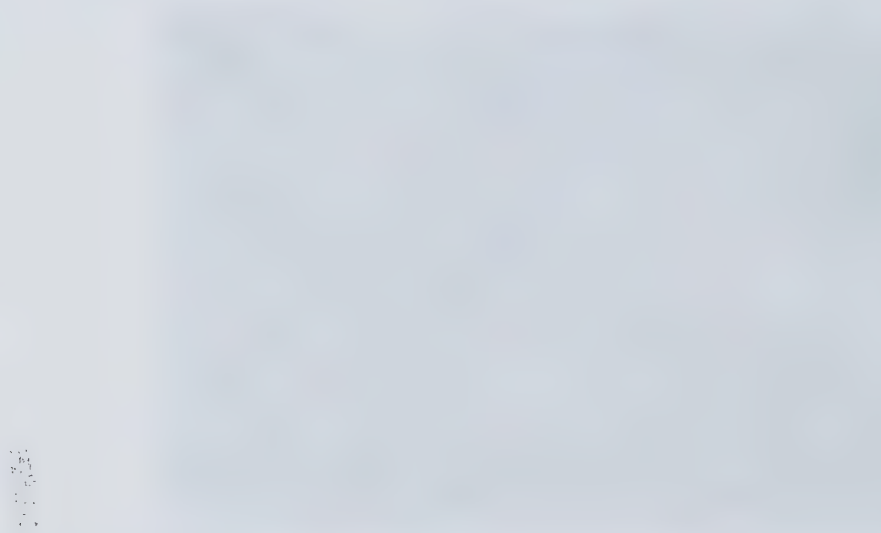
Slice transversely, taking a section at mid-spleen.

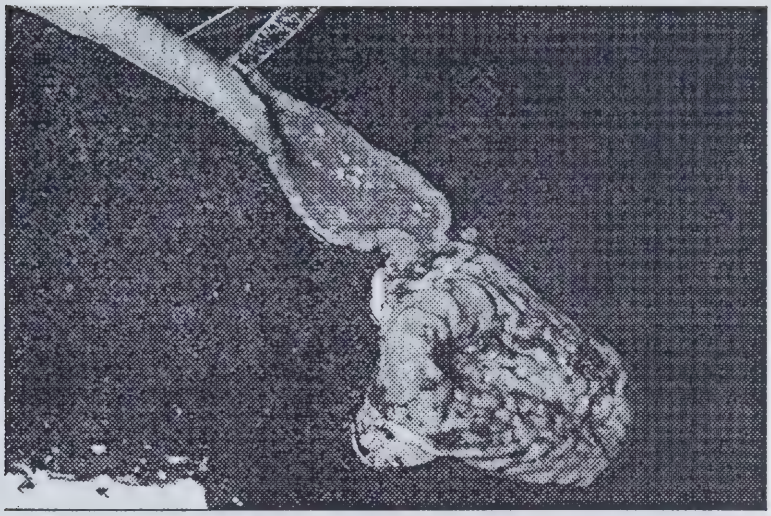
(8). Incise and examine the pancreas, then take sections of both ends.



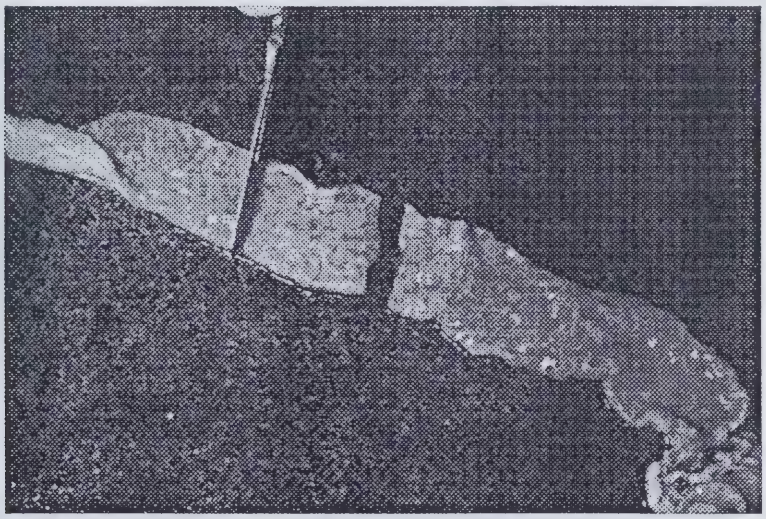
(9). The intestinal tract mesentery should be examined for parasites and then



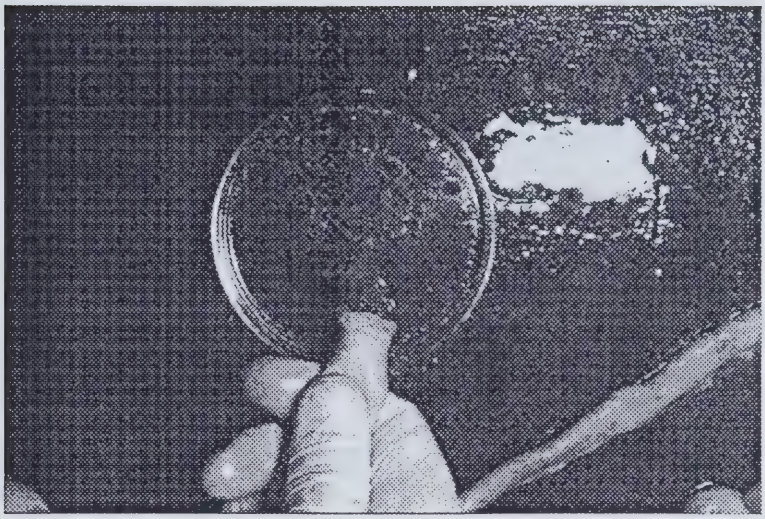




opened to allow the intestinal lumen to be examined. Samples of tied-off intestine which contain fecal bulges should be obtained at this time.

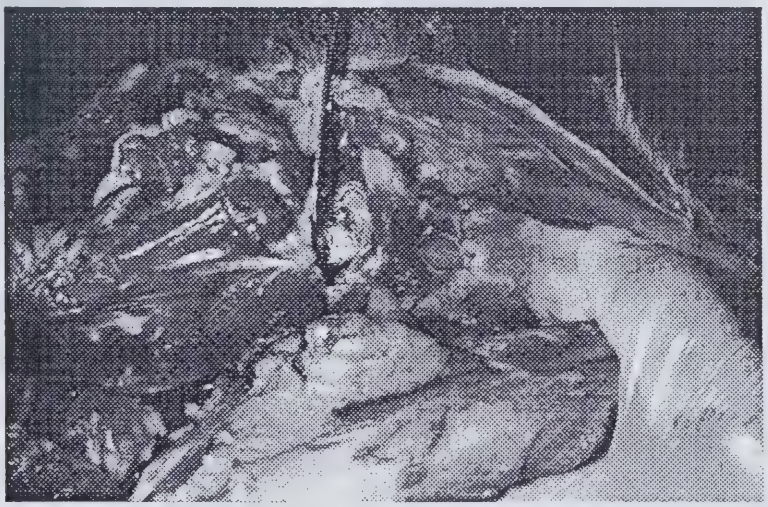


Samples for *Salmonella* sp culture are obtained from small intestine.

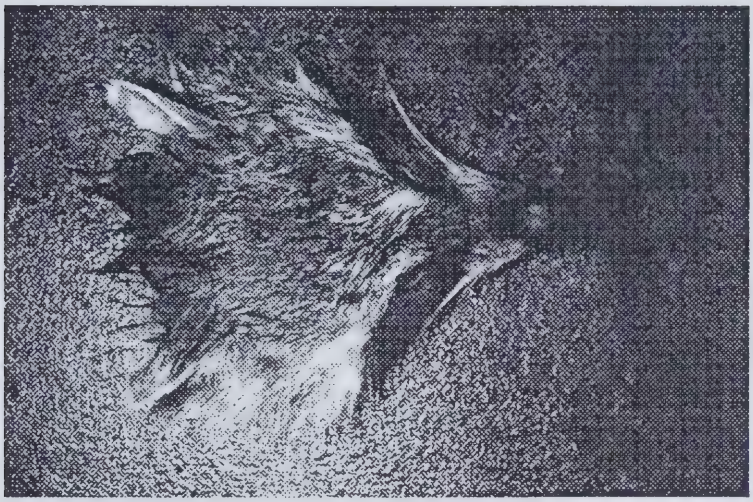


Parasitology specimens are to be obtained from the large intestine. These are not formalized and are placed in Whirl-paks. Then, starting at the stomach, open the entire length of the intestine, inspecting as you go. Sections should be taken from the small and large intestines, including a mesenteric lymph node, and placed in formalin.

h. Collection of the brain (only in cases when CNS signs are present)

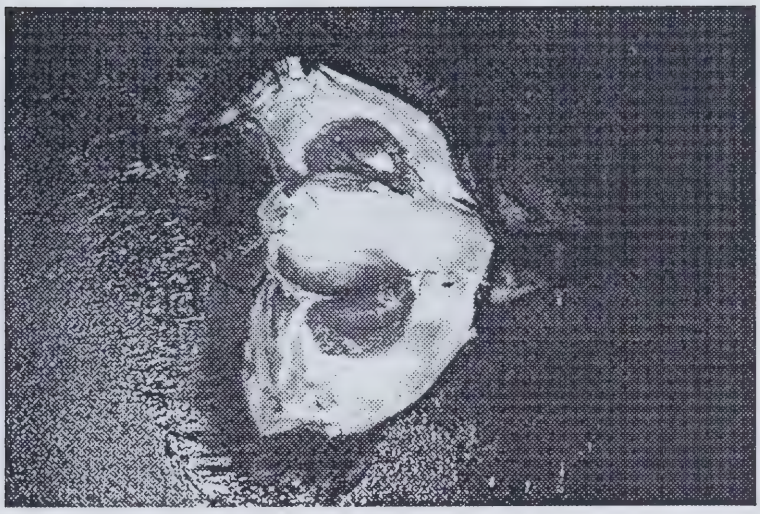


(1). Disarticulate the atlanto-occipital joint by approaching from the ventral aspect and

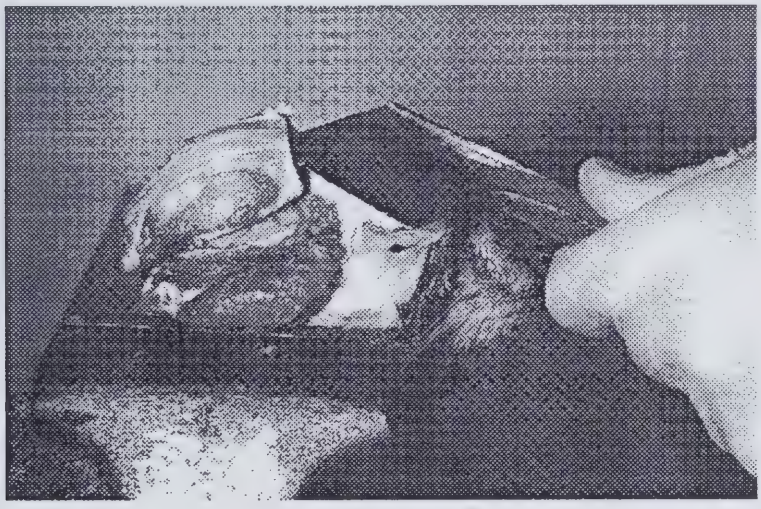


removing the head.

(2). Reflect the skin covering the skull, including the eyelids, to facilitate removal of the eyes and brain.

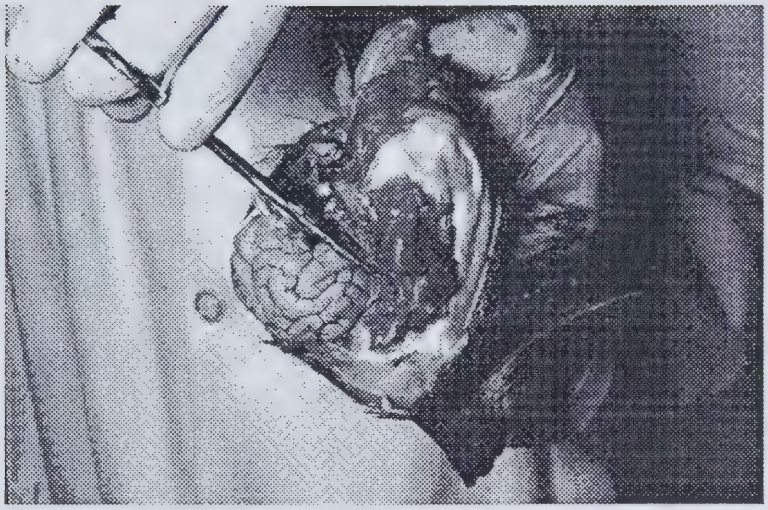


(3). Reflect the muscles downward from the top of the head.



With a chisel remove the dome of the skull beginning at the spinal cord outlet, cutting forward along a line indicating the lateral margins of the cerebral hemispheres extending to a line connecting the eyes. Cut the frontal bone transversely on a line connecting the eyes.

(4). With a T-bar reflect the skull cap. Using scissors cut away the meninges covering the brain.



Invert the head to gain gravitational pressure which will pull the brain out of the cranium, then incise the cranial nerve roots and ventral attachments and remove the brain.

(5). Divide the brain in half by cutting along the mid-sagittal plane and place one half in fixative. The second half is halved again sagittally and half of this section placed in a Whirl-pak for state testing. The remaining half is placed in a Whirl-pak for in-house testing.

(6). NOTE: In the absence of proper materials a whole head may be submitted. Remove head as above and double bag.

VII. APPENDICES

1. Appendix A - This is a quick reference for the preservation and collection techniques specified in the necropsy manual.

   a. Sterile technique

   Sterile technique is the process in which one collects specimens for culture in such a way that microbial contamination is minimized or eliminated. The premise is that when the sample is collected it should come into contact with no non-sterile surfaces or instruments before it is placed in a sterile container for submission. This is critical when culturing, as contamination will confuse the culture tests. When obtaining fresh tissues for submission for culture the best tissue to use is that which has not been exposed to the external environment. Therefore, when sampling organs follow these steps:

   (1). Choose a site on the organ for sampling. If the organ is diseased take tissue from a lesioned site if possible.

   (2). Using a pre-sterilized scalpel and tweezers, or ones that have been flamed until red hot and allowed to cool without contacting a non-sterile surface, remove the tissue to be cultured. A piece no smaller than 2.5cm x 2.5 cm should be obtained.

   (3). Place the tissue sample immediately into a sterile container for refrigeration and submission without allowing it to touch any non-sterile surfaces.

   b. Fixing tissues in formalin

   (1). Use a clean scalpel to cut sections 0.5 cm thick (with cut surfaces parallel, bread loaf fashion) and broad enough to orient and identify the organ (2-3 cm across). The sample should contain both diseased and adjacent normal portions of the organ when available and applicable. If collecting a larger sample of tissue is necessary, make slices across the sample at 0.5 cm intervals, then proceed with the normal fixation steps, except with brain tissue. Do not make slices through the brain.

   (2). Place the tissues and the entire brain are placed in 10% formalin. The volume of formalin should be about 10 times that of the tissue.

   (3). Each specimen must have its own label. The label should have on it, written in waterproof marker, an identification number and the type of tissue sampled.

   (4). After the tissues have been in formalin for 24-48 hours, pour off the formalin and rinse the tissues in fresh water. Replace the specimens and labels in a new solution containing 10% formalin. Histopathologic staining results are greatly improved by performing this last step.

   The following tissues should be collected:
   Peripheral lymph nodes
   Lung (left apical lobe, right diaphragmatic lobe)
   Heart (opened heart)
   Tonsil

Stomach (only if abnormality is present)
Intestine (small with mesenteric lymph node and large)
Pancreas
Liver
Kidney
Thyroid
Adrenals
Spleen
Brain (only half is fixed, other half is frozen)

c. Preserving gross parasites

Gross specimens require fixation and preservation prior to mailing. Tremetodes and cestodes that are recovered at necropsy or passed in the feces should be placed in tapwater, kept overnight in a refrigerator, and then fixed and stored in 10% formalin. Nematodes should be fixed, preferably in 70% ethanol, but can also be fixed in 10% formalin. Most nematodes from domestic animals can be easily identified, but if nematodes are from exotic or wild animals, special fixation is needed. The best method is to heat 70% ethanol to about 60°C, drop the parasites into the hot alcohol one by one, and remove them as soon as they are fixed in an extended position. They should then be placed in fresh 70% ethanol for mailing.

Ectoparasites should be fixed and submitted in 70% ethanol.

Look for these parasites:
Ticks
Mites
Fleas
Lice
Lungworms (*Nematoda* sp)
Heartworms (*Nematoda* sp)
Stomach Worms (*Nematoda* sp)
Pancreatic flukes (*Trematoda* sp)
Liver flukes (*Trematoda* sp)
Intestinal parasites (*Cestoda* sp and *Acanthocephala* sp)

d. Fecal Collection

Parasites to be characterized by:

Flotation:
Baylisascaris procyonis
Macracanthorhynchus ingens
Atriotaenia procyonis
Mesecestoides

Molineus barbatus
Physaloptera rara
Capillaria plica (also urine)
Capillaria procyonis (also tongue digests in strat.squam. epith.)
Paragonimus kellicotti
Sarcocystis sp (also tissue)

Sedimentation:
Eurytrema procyonis (can also be seen in pancreatic ducts)
Paragonimus kellicotti
Crenosoma globei

One of two methods may be used, depending on availability of fixing agents and time constraints.

(1). For in-field necropsy with limited supplies:
(a) Locate a section of fresh small intestine that has not been cut or emptied of its contents and that appears to contain feces.
(b) Using suture material, tie off a 5 cm portion of small intestine at both ends to catch all contents within the tied-off section.
(c) Cut out the tied-off section and place it in a Whirl-pak. Keep the Whirl-pak chilled. This sample is for *Salmonella* sp culture and must reach the micrbiology lab immediately.
(d) For parasitology, repeat the above procedure with a section of large intestine. If fecal balls are not present in the large intestine, another sample from the small intestine may be submitted. Take this sample from the section closest to the large intestine.

(2). For most accurate testing:
Fecal samples for parasitology should be mailed in plastic bags or other water -tight containers. If the samples can reach the laboratory in three days, no preservatives need to be used. However, some of the ova may hatch during this time unless air is excluded from the container. The recommended method is to place the fecal sample in 10 to 15 volumes of 10% formalin (the same concentration for submitting histopathology samples) or, if coccidia are suspected, place the feces in 2.5% potassium dichromate solution. If special procedures such as the Baermann technique to find *Strongyloides spp.* or lungworm larvae are needed, they should be requested. Fresh fecal samples without preservatives or fixatives are required for the Baermann technique.

If examination for intestinal protozoa other than coccidia (particularly *Giardia* sp or amoebae) is requested, the sample should be fixed and shipped using sodium acetate-acetic acid-formalin (SAF) fixative. The fixative also preserves helminth eggs. The SAF solution is prepared as follows:

Sodium acetate	1.5 g
Acetic acid, glacial	2.0 ml
Formaldehyde solution (40%)	4.0 ml
Water	92.5 ml
Total	100.0 ml

Mix one volume of feces with at least three volumes of SAF fixative. Shake the container well to ensure complete dispersal of the specimen. Please call or write to receive vials containing SAF fixative from the Parasitology Laboratory.

e. Tissues for toxicology

Tissue samples collected for toxicology must be frozen. A heavy metal assay will be run on these tissues.

Collect samples of 50 -150gm from each of the tissues and organs listed below. Samples collected for the heavy metal assay may be stored in Whirl-pak bags and frozen.

Specimen	Quantity	Comment
Liver	150 grams	Avoid gallbladder
Kidney	150 grams	Submit cortex and medulla
Fat	50 grams	

2. Appendix B - This appendix contains the formulae for fixatives and preservatives mentioned in appendix A.

10% Formalin formulation

Commercial formaldehyde (37-40%)	100ml
Water	900ml

70% Ethanol formulation

Ethyl alcohol (95%)	735ml
Water	265ml

3. Appendix C - This appendix contains the addresses for submitting samples.

Laboratories of Veterinary Diagnostic Medicine - Urbana
University of Illinois

College of Veterinary Medicine
P. O. Box U
2001 South Lincoln Avenue
Urbana, IL 61801

Dr. Laura L. Hungerford
Dr. James F. Zachary

Working Hours: Monday through Friday – 8 a.m. to 5 p.m.
Saturdays and Holidays – mail pickup only

4. Appendix D -This appendix outlines the sample types for various tests and materials required for submission.

Sample and Test Types Per Disease

DISEASE	SAMPLE TYPE
A. Protozoal	
Toxoplasmosis	serum, feces
B. Bacterial	
Leptospirosis	serum, urine, kidney tissue
Brucellosis	serum
Salmonellosis	lymph node, spleen, liver, small intestine
C. Viral	
Parvovirus	serum, feces
Pseudorabies	serum, brain, tonsil
Distemper	serum, brain
Rabies	brain

Container Type Per Test and Sample

SAMPLE	TEST	MAILER	SUPPLIER

Other Supplies and Suppliers:

Central Stores	
Dissection Scissors, Small	35 54 4500
Dissection Scissors, Large	35 54 5400
Ethanol (100%, 1pint)	34 09 2400
Needles (1in 20GA.)	35 51 6900
Petri dishes (100 x 15mm)	37 43 2800
Scale (platform, triple beam)	35 03 1400
Scalpel Handels	35 48 7700
Syringes (10 ml)	32 68 8200
Tweezers (forceps)	
curved	35 39 4000
small	35 39 6000
toothed	35 39 5500
Koch Supplies, Inc.	
5'' Sharp Skinning Knife	01 00 90
Whetstone	01 08 02
Mettam Safety Equipment	
Rubber Gloves (sizes 7,8,9)	390
Surgipath	
Formalin (100% buffered, 0.5gal)	BF 6400

Suppliers Addresses:

Baxter Scientific
1210 Waukegan Rd.
McGraw Park, IL 60085
1-800-942-4591

Central Stores and Receiving
200 Central Receiving Building
1609 South Oak Street
Champaign, IL 61820

Koch Supplies, Inc.
1411 W. 29th St.
Kansas City, Mo. 64108
1-800-821-7787

Mettam Safety Equipment
3817 N. Vermilion
Danville, IL 61832
1-800-252-3009

Nasco
901 Janesville
Fort Atkinson, WI 53538
1-800-558-9595

Surgipath
301 Zeigler
Box 769
Grayslake, IL 60030

VIII. GLOSSARY OF TERMINOLOGY

adult -	An animal greater than two years of age.
ante-mortem -	Occurring before death.
apex -	The point or extremity of a cone.
apical -	Pertaining to the apex.
autolysis -	Breakdown of cells or tissue by self produced enzymes.
caudal -	Toward the tail.
cranial -	Toward the head.
dorsal -	Toward the spine or back of the body.
in situ -	In the natural or original position.
juvenile -	An animal less than one year of age.
lateral -	Away from the midline of the body.
medial -	Toward the midline of the body.
meninges -	Referring to the membranes covering the brain and spinal cord.
pectoral -	Coming from the chest.
post-mortem -	Occurring after death.
putrefaction -	The decomposition and breakdown of organic matter by bacteria and fungi.
sagittal -	The median plane of the body, and any plane parallel to this.
sterile technique -	The method in which samples are obtained.
yearling/subadult -	An animal greater than one year, but less than two years of age.
zoonotic -	Disease communicable from animals to man under natural conditions.

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Field Necropsy Form (Raccoon Project) Date: _____ Time: _____

Submitted by: _____

Location found: _____
County City/Town Address ZipCode

☐ Dead
☐ Alive
☐ Female
☐ Male
☐ Juvenile
☐ Yearling
☐ Adult
☐ Emaciated
☐ Thin
☐ Normal
☐ Fat

Nervous Signs
☐ Yes
☐ No
☐ Unknown

Respiratory Signs
☐ Yes
☐ No
☐ Unknown

Gastrointestinal Signs
☐ Yes
☐ No
☐ Unknown

Ectoparasites
☐ Yes
☐ No

Trauma
☐ Yes
☐ No

Post-mortem Condition
☐ Good
☐ Fair
☐ Poor

Dentition
☐ Good
☐ Fair
☐ Poor

Weight _____
Length _____

Primary incision
☐ lymph node
☐ liver (culture)
☐ kidney (culture)
☐ spleen (culture)

Thoracic pluck
☐ thyroid
☐ parathyroid
☐ heart
☐ lung
☐ lymph node

Abdominal pluck
☐ adrenal
☐ kidney
☐ liver
☐ pancreas
☐ duodenum
☐ jejenum
☐ ileum
☐ colon
☐ spleen

Head
☐ brain

Toxicology
☐ liver
☐ kidney
☐ stomach content
☐ brain
☐ urine
☐ body fat
☐ heart blood

Leptospirology
☐ kidney

Virology
☐ liver
☐ kidney
☐ spleen
☐ lung
☐ lymph node
☐ duodenum
☐ jejenum
☐ ileum
☐ colon
☐ brain

Bacteriology
☐ liver
☐ kidney
☐ spleen
☐ lung
☐ lymph node
☐ duodenum
☐ jejenum
☐ ileum
☐ colon
☐ brain

Parasitology
☐ liver
☐ kidney
☐ spleen
☐ lung
☐ lymph node
☐ duodenum
☐ jejenum
☐ ileum
☐ colon
☐ brain

Comments:

Field Necropsy Form (Raccoon Project) Date: _____ Time: _____

Submitted by: _____

Location found: _____

County City/Town Address ZipCode

☐ Dead
☐ Alive
☐ Female
☐ Male
☐ Juvenile
☐ Yearling
☐ Adult
☐ Emaciated
☐ Thin
☐ Normal
☐ Fat

Nervous Signs
☐ Yes
☐ No
☐ Unknown

Respiratory Signs
☐ Yes
☐ No
☐ Unknown

Gastrointestinal Signs
☐ Yes
☐ No
☐ Unknown

Ectoparasites
☐ Yes
☐ No

Trauma
☐ Yes
☐ No

Post-mortem Condition
☐ Good
☐ Fair
☐ Poor

Dentition
☐ Good
☐ Fair
☐ Poor

Weight _____
Length _____

Primary incision
☐ lymph node
☐ liver (culture)
☐ kidney (culture)
☐ spleen (culture)

Thoracic pluck
☐ thyroid
☐ parathyroid
☐ heart
☐ lung
☐ lymph node

Abdominal pluck
☐ adrenal
☐ kidney
☐ liver
☐ pancreas
☐ duodenum
☐ jejenum
☐ ileum
☐ colon
☐ spleen

Head
☐ brain

Toxicology
☐ liver
☐ kidney
☐ stomach content
☐ brain
☐ urine
☐ body fat
☐ heart blood

Leptospirology
☐ kidney

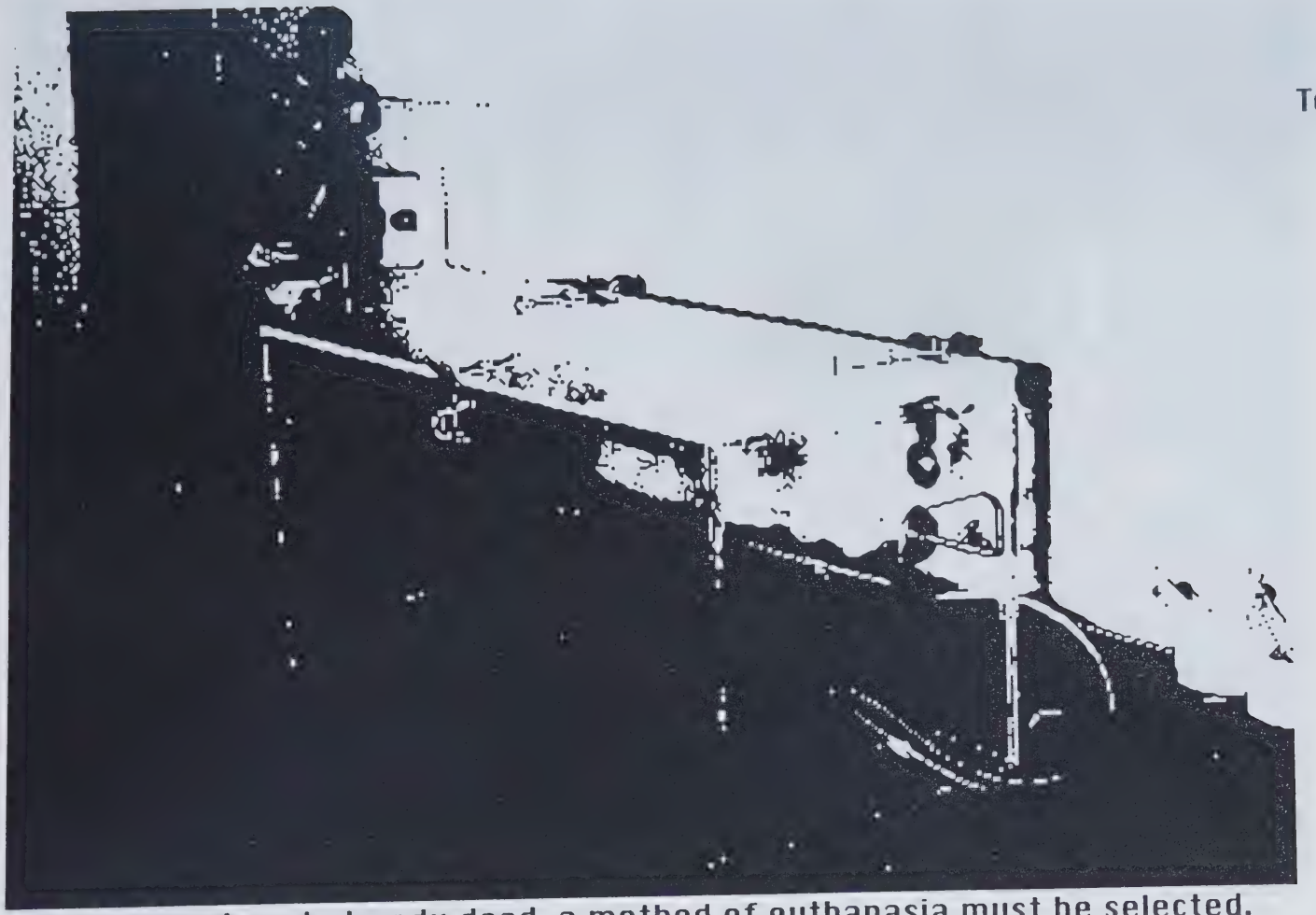
Virology
☐ liver
☐ kidney
☐ spleen
☐ lung
☐ lymph node
☐ duodenum
☐ jejenum
☐ ileum
☐ colon
☐ brain

Bacteriology
☐ liver
☐ kidney
☐ spleen
☐ lung
☐ lymph node
☐ duodenum
☐ jejenum
☐ ileum
☐ colon
☐ brain

Parasitology
☐ liver
☐ kidney
☐ spleen
☐ lung
☐ lymph node
☐ duodenum
☐ jejenum
☐ ileum
☐ colon
☐ brain

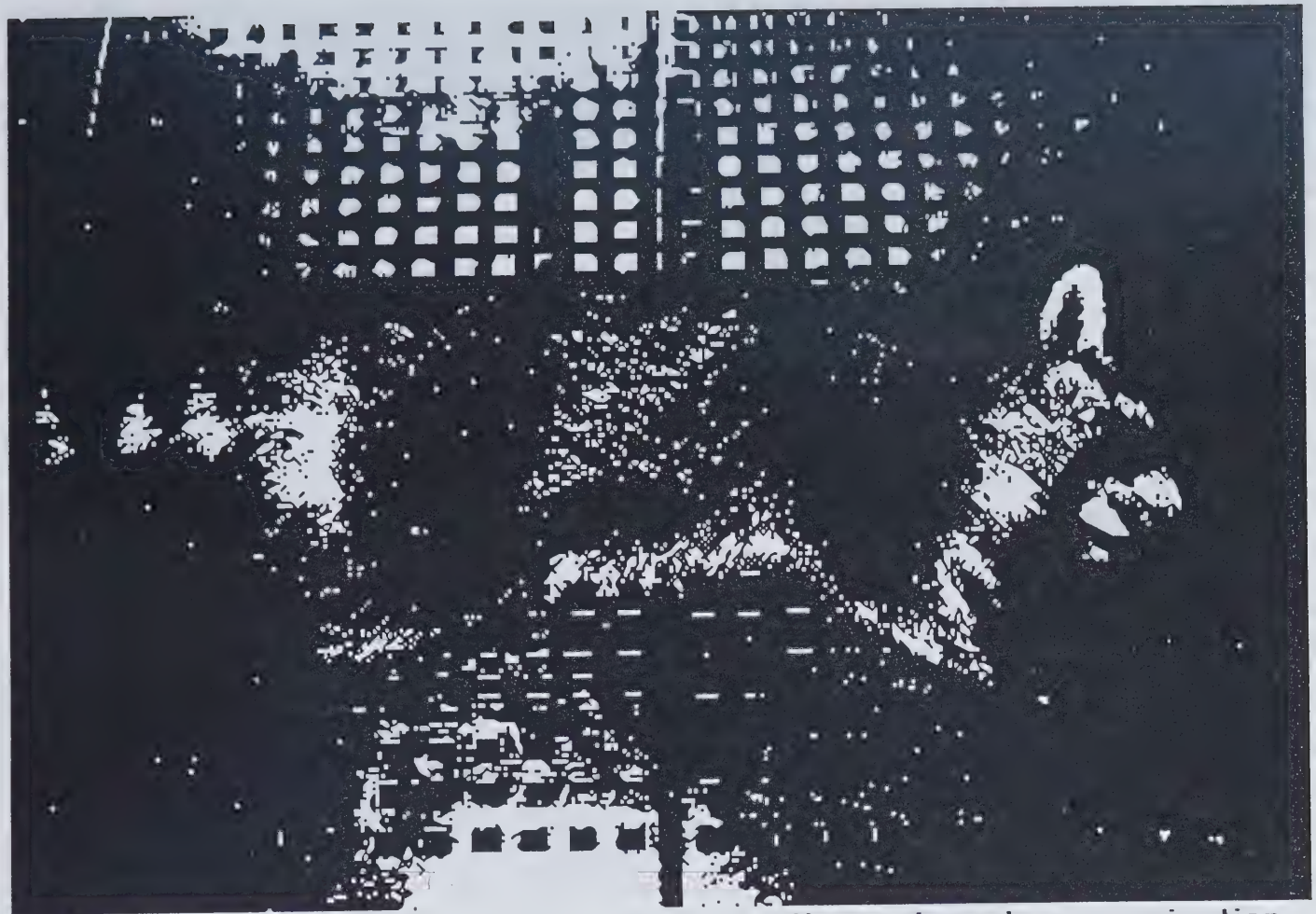
Comments:

Raccoon
Necropsy
Techniques 1.0



f the raccoon is not already dead, a method of euthanasia must be selected.
ne method selected must not interfer with the collection of tissues for
nalyses (ie: bullet in brain-rabies examination). An approved and humane
ıethod of euthanasia should be used.

Cont



Raccoon
Necropsy
Techniques 1.0

he raccoon should be placed on its left side for the post mortem examination. he animal's nutritional and post mortem condition, approximate age, sex, and ealth status should be determined at this time.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

:xamine the oral cavity and then the teeth for decay and tartar. Make sure
jou wear protective clothing and gloves to prevent the possible transfer of
:oonotic diseaseS (ie: rabies, leptospirosis, etc) to you during the post mor'tem
:xamination.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

he skin should be palpated for abcessess and tumors. The presence of
ectoparasites such as ticks, lice, and fleas should be noted and samples
collected for identifcation if appropriate. Examine the anus for signs of
diarrhea, a common finding in parvoviral enteritis.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

se a sharp scapel, knife, or razor blade to cut the skin under the right
orelimb. If little or no blood flows from the severed blood vessels, the animal
ıay have lost a lot of blood due to internal bleeding, parasites, or other
isease problems. If the blood, appears very watery (few red blood cells), this
nimal may be anemic due to chronic disease, parasitism, or malnutrition.

Cont

Back

xamine the internal and external surfaces of the ears for ticks. Save samples
f the ticks for identification because of their role in the transmission of Lyme
lisease and other infectious agents.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ontinue widening and deepening the incision until you can push the right orelimb onto the surface behind the raccoon.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

lake the same type of incision in the skin where the right hindlimb joins the
nguinal area. Cut through the skin and muscles to exposure the hip joint. The
ip joint must be cut open and the ligament between the head of the femur
nd the socket of the hip joint must be cut in order to have the hindlimb lay
lat on the table behind the raccoon.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ontinue widening and deepening the incision until you can reflect the right
indlimb onto the surface behind the raccoon. Cut the skin away from the
nuscle (skin the raccoon) to expose the abdominal muscles and the rib cage.

Cont

Back

ut the abdominal muscles along the caudal border of the ribs; extend the cut long the back; and then cut the abdominal muscles along the caudal edge of ıe pelvis. Pull this triangular flap of muscle downward to expose the bdominal organs.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

Jith a knife, make a small cut in the diaphragm. Listen for air entering the
hest cavity as the lungs collapse. Cut the ribs with a pruning shears from
he caudal edge, along the sternum, to the cranial edge of the rib cage.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

epeat the process by cutting the ribs along the back. Remove the rib cage to
xpose the thoracic organs.

Cont

Back

epeat the process by cutting the ribs along the back. Remove the rib cage to
xpose the thoracic organs.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

f samples are to be taken for bacteriologic evaluation, they must be collected efore the tissues are handled to minimize contamination. Examine the rgans in both the thoracic and abdominal cavities and if appropriate collect terile samples for additional tests. Note the presence or absence of fluid in ither cavity and lesions affecting any organ system.

Cont

Back

Raccoon Necropsy Techniques 1.0

› better examine the lungs and the heart, they need to be removed from the ıoracic cavity. To remove these organs, first remove the skin from the entral neck and chin to exposure the lower jaw. Cut the flap of muscle that ridges between each side of the lower jaw and reach into the mouth and pull ıe tongue out through the lower jaw.

Cont

Back

ontinue to pull caudally on the tongue and cut any attachments to the roof
nd sides of the oral cavity.

Cont

Back

ontinue to pull the tongue caudally and cut the muscular and cartilagenous ttachments between the larynx, a cartilagenous box-like structure located ear the base of the tongue, and the oral cavity.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

hen the larynx is free, you can examine the openings into the trachea and
sophagus. Continue to cut the attachments between these structures and
ıe neck while pulling caudally on the tongue.

Cont

Back

ıfter cutting the attachments to the neck, the trachea and esophagus will be ree. Continue to cut the attachments of these structures down to the area vhere these structures enter the thoracic cavity.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

t the entrance to the thoracic cavity, cut the attachments and pull the lungs nd heart from the cavity. You will need to cut the mesenteric attachments nd the aorta, vena cava, and esophagus, caudally, where they pass through he diaphragm.

Cont

Back

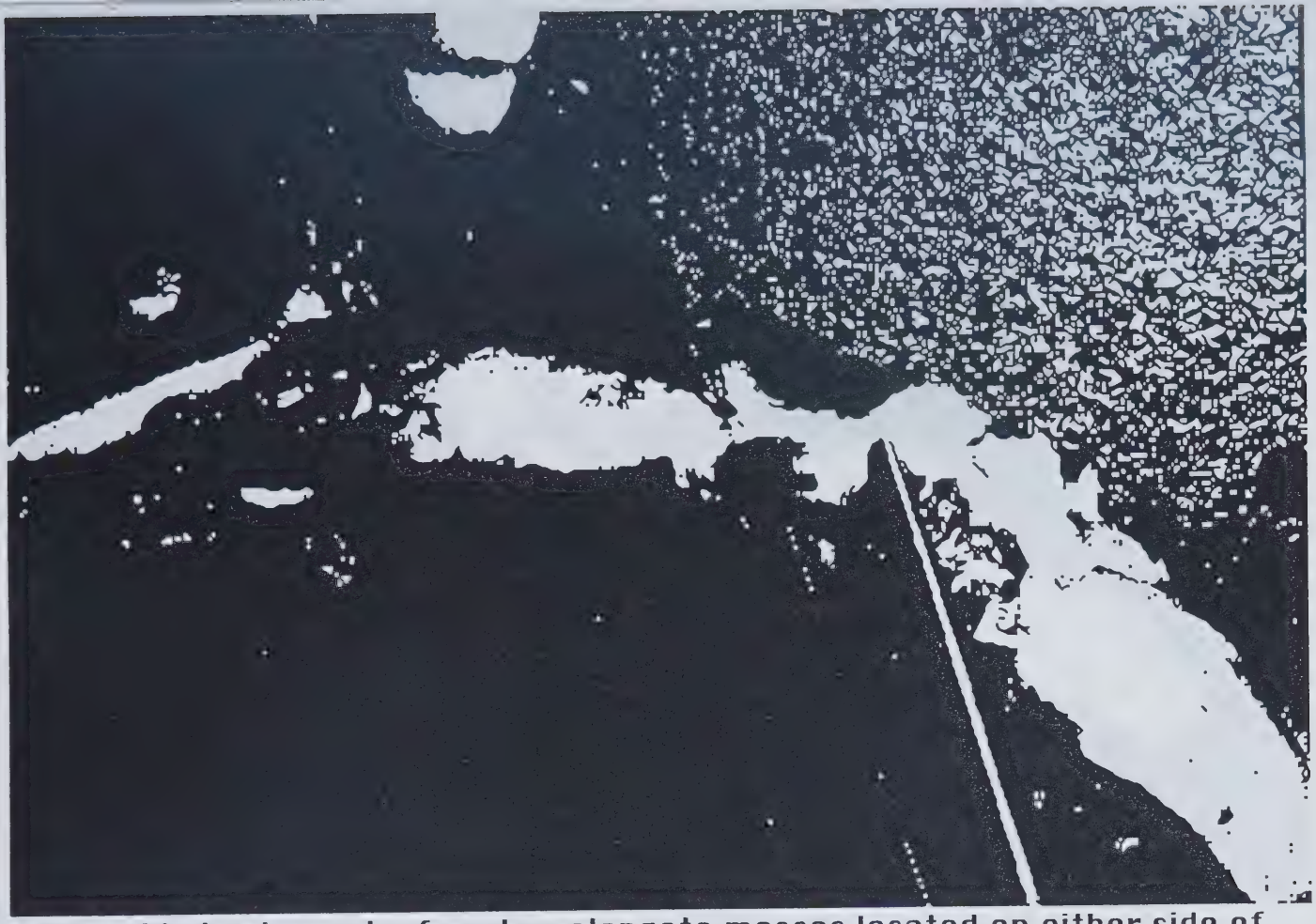
Raccoon
Necropsy
Techniques 1.0

lace the lungs on a flat surface and examine all structures for lesions and in
articular the lungs for evidence of pneumonia or parasitism. Normal lungs
ave a pink color and are soft and spongy when palpated. Pneumonic lung will
e firm, have a tan to brown color, and will be confined to the anterior and
entral aspects of the lung field. Pneumonia is seen in some raccoons with
istemper.

Cont

Back

Raccoon
Necropsy
Techniques 1.0



ıe thyroid glands can be found as elongate masses located on either side of ıe trachea just behind the larynx. Note their presence. They do not need to e sampled.

Cont

Back

ind the opening of the esophagus above the larynx. The esophagus is the
nick muscular tube associated with the trachea and larynx. Place a knife or
cissors in this opening and cut the esophagus caudally.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ext, locate the opening of the larynx, which is a cartilagenous box-like
tructure located near the base of the tongue, and cut through its dorsal
spect with a sharp knife or scissors.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ontinue this cut through the dorsal roof of the trachea, caudally, until you
each the separation of the trachea into branches within the lung. The trachea
as stiff cartilagenous rings that prevent it from collapsing. Examine the
pened surface of the trachea for ulcers and parasites.

Cont

Back

pen several of the branches of the trachea and follow them deep into the
ıng. Look for parasites and evidence of pneumonia. Pneumonia maybe
ıdicated by a yellow-white granular exudate mixed with blood in the airways.

Cont

Back

ake samples of the lung for evaluation at this time. Before cutting the lung
ito transverse sections (bread-loaf slices), examine its surface and palpate
ne tissue for nodules and firmness. Lungs with parasites may have abcesses
i the caudal (diaphragmatic) lobes.

Cont

Back

nd and open the pericardium; the covering the surrounds the heart. Note the
olume, color, and consistency of fluid present in this space.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ext examine the heart. Open the heart following a path that the blood would
ow through the heart. Examine the valves and blood vessels associated with
oth the right and left atria and ventricles. Note the thickness of each
uscular wall that forms each chamber and the volume of each chamber.

Cont

Back

ıe right ventricle demonstrated here has thin muscular walls and a large
hamber. The left ventricle has thick muscular walls and a smaller chamber.
ıe valve leaflets should be delicate, shiny, and slighty opaque.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ets now return and examine the abdominal cavity and its organs. Look and
lentify the stomach, small intestines, large intestines, kidneys, and bladder
efore removing the organs and examing each structure.

Cont

Back

ıe adrenal glands can be found as small elongate to bean-shaped masses
ıcated just cranial and medial to the anterior edge of each kidney. It is best
ɔ locate and sample them at this time. After removal of the organs from the
bdominal cavity they will be difficult to find.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

n one hand grab all of the abdominal viscera including the small intestine,
idneys, liver, and spleen. Pull this mass of organs caudally and cut them
way from their attachments with the diaphragm and mesentery associated
ith the back-bone. Be careful not to cut into the intestines, so the contents
o not leak out.

Cont

Back

ıntinue to pull the visceral organs and mesenteries caudally and cut the
tachments to the abdominal surfaces until the colon is the only remaining
tachment.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ıt the attachment to the colon, as far caudally as possible, to free the
ıdominal ɒiscera.

Cont

Back

ssect the spleen, liver, and kidneys free from the abdominal viscera. Look to
e if these organs are of normal size, shape, color, and texture. Examination
d sectioning of these organs can be made easier if they are placed on a hard
rface such as a cutting board.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

t the liver in serial sections and examine the cut surfaces for lesions.
rmal liver should have a tan-brown to mahogeny color. A modest amount of
ıod should come out of cut surfaces. Lesions in ther liver could consist of'
ıite spots of varied sizes and larger areas called abcessess that contain
ck white-yellow fluid .

Cont

Back

Raccoon
Necropsy
Techniques 1.0

t the mesentery away from the kidneys and check, if not located earlier, for
e presence of adrenal gland in the mesentery just anterior and medial to
ch kidney. You need only to identify the presence or absence of both
renal glands.

Cont

Back

ce each kidney in a longitudial plane and examine for lesions. Normal kidney
ll be red-brown to mahogeny color. Lesions in the kidney would most likely
associated with Leptospirosis and could include white to pale tan streaks
xed with areas of hemorrhage.

Cont

Back

xt, examine the mesentery for lesions. It should be translucent. If the
ccoon has peritonitis (inflammation of the abdominal cavity), the mesentery
ay be opaque and covered with white to yellow granular material (exudate)
d white strands (fibrin).

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ıd the pancreas which is located at the curvature between the stomach and
odenum. It is a pale pink organ that has a lobular appearance. Examine it
r lesions; it is unlikely that the pancreas will have lesions. Cut the stomach
d duodenum away from the mesentery.

Cont

Back

ntinue cutting the remaining small intestines and large intestines away
ɔm the mesentery.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

e entire digestive system is now ready for detailed examination.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ıueeze feces from the colon and place them in a petri dish or other container
ır parasite analysis.

Cont

Back

ɔen the stomach starting from the esophageal opening. Extend the incision
the pylorus.

Cont

Back

en the stomach starting from the esophageal opening. Extend the incision
the pylorus.

Cont

Back

:amine the contents of the stomach and look for lesions (ulcers) on or
ırasites (Physaloptera sp.) attached to the surface of the stomach. The most
ımmon parasites of the stomach are Physaloptera sp.

Cont

Back

ıt through the pylorus and enter the duodenum. The duodenum will often
ıntain bile, a brilliant yellow to yellow-green pigment.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ontinue to open the duodenum. Select a specimen for histologic and/or
acteriologic analyses by cutting out a section. Samples are obtained for
acteriology by tying off a loop of small intestine and placing it in a petri dish
or submission. Open and sample the remaining digestive tract in a manner
milar to that described above.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

e brain will have to be removed for analysis. Locate the occipital-atlantal
int, the joint that connects the head to the spinal column.

Cont

Back

t through the muscle and ligaments that cover this joint and expose the
inal cord. Cut through the spinal cord, remaining portions of the joint, and
e dorsal musculature and skin to remove the head.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ke the head and place it on a hard flat surface. It is often easier to place
e entire head in a zip-lock bag and send it properly packaged and labeled to
e laboratory for appropriate analyses (rabies, distemper), then to remove
e brain and send it in.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ıt the skin and subcutaneous connective tissue from between the eyes
ıudally back to the neck.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

eflect the skin to either side by cutting the subcutaneous tissue under the
in.

Cont

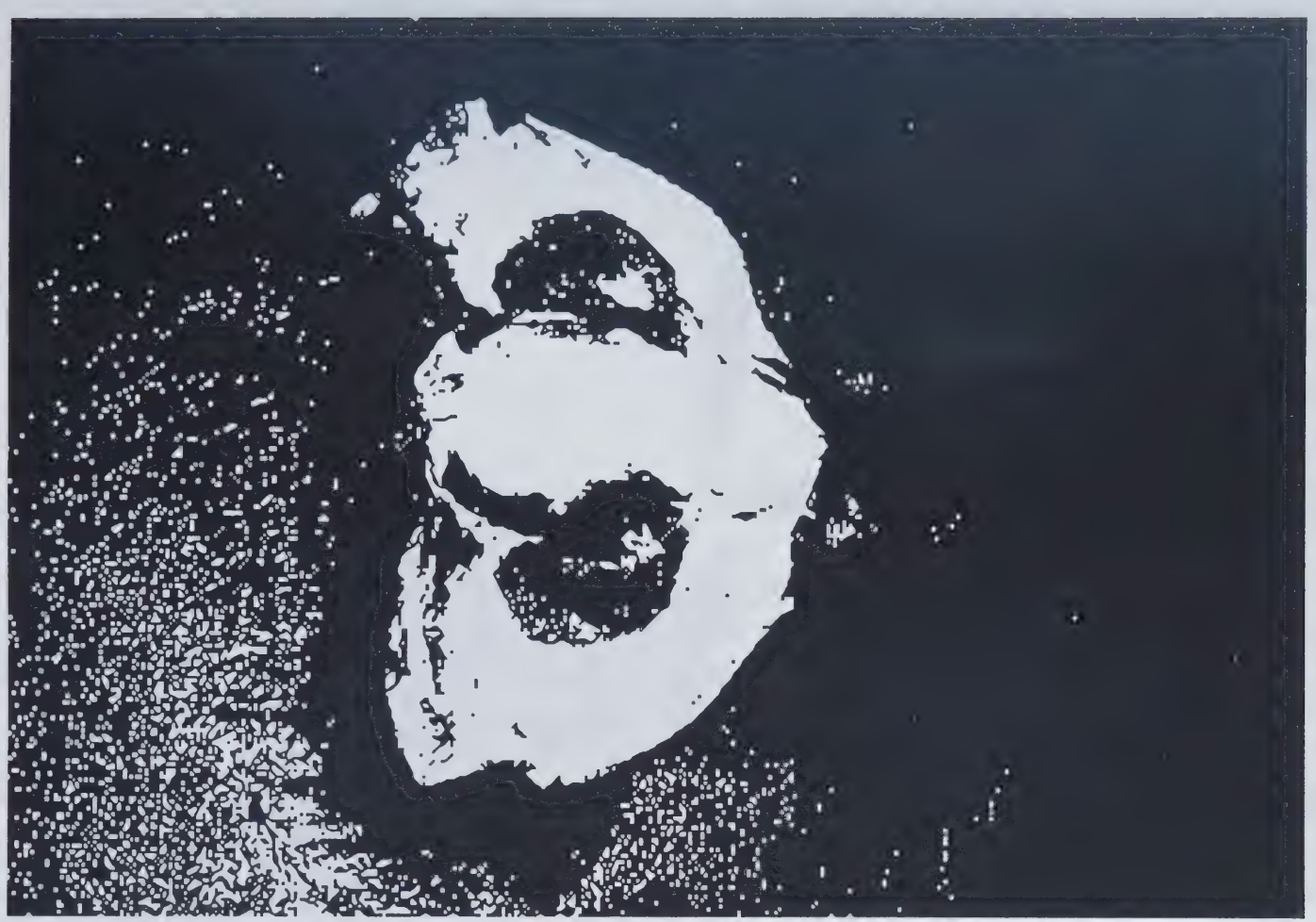
Back

Raccoon
Necropsy
Techniques 1.0

ɔcate the muscle on either side of the head.

Cont

Back



Raccoon
Necropsy
Techniques 1.0

ut through this muscle, starting dorsally, until it can be reflected away from
ıe skull.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

Using a hammer and chisel, make a transverse cut through the skull behind the eyes. Extend the cut down each side and then caudal until the cuts on each side connect with the occipital joint at the foramen magnum (opening into the skull).

Cont

Back

Raccoon
Necropsy
Techniques 1.0

se the chisel to pull the skull away from the brain in a cranial to caudal
anner.

Cont

Back

Raccoon
Necropsy
Techniques 1.0

ıe entire skull must be removed. Pull the skull away from the brain and cut
ll attachments to brain, muscle, or bone.

Cont

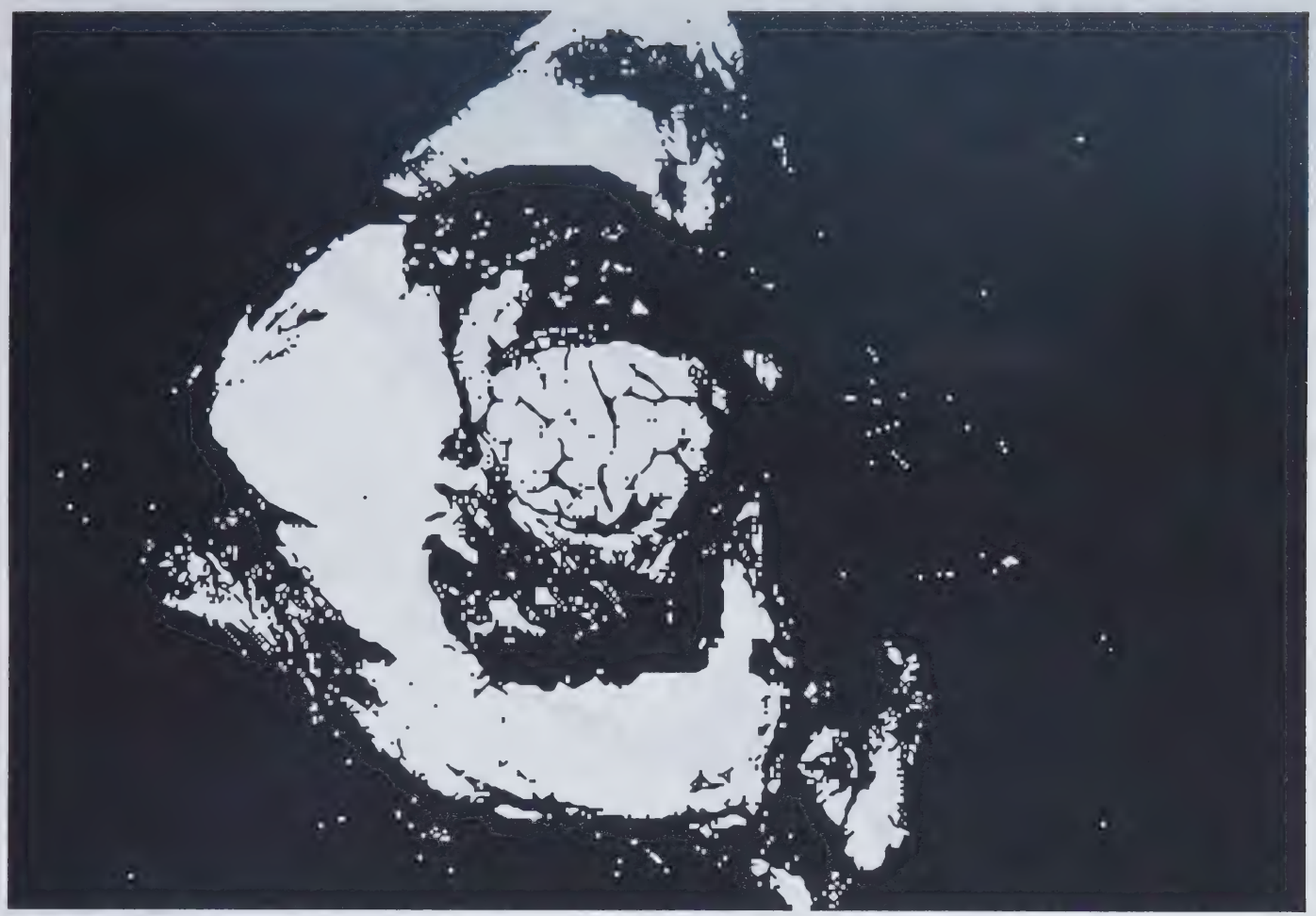
Back

Raccoon
Necropsy
Techniques 1.0

se a sharp knife or scissors to cut through the outer dense covering of the rain (dura mater).

Cont

Back



Raccoon
Necropsy
Techniques 1.0

emove the dura mater from both sides of the brain so the brain can be
emoved easily.

Cont

Back

old the head upside-down. Cut the attachments such as nerve roots and eninges along the floor of the brain. The brain will begin to fall-free from ıe skull. It will fall out once most connections are severed.

Cont

Back

ıt the brain in half and use one half for additional testing as required
istemper and rabies) and the other half for fixation and histologic analysis.

Done

Back

Useful Terminology:

adult - An animal greater than two years of age.
ante-mortem - Occurring before death.
apex - The point or extremity of a cone.
apical - Pertaining to the apex.
autolysis - Breakdown of cells or tissue by self produced enzymes.
caudal - Toward the tail.
cranial - Toward the head.
dorsal - Toward the spine or back of the body.
in situ - In the natural or original position.
juvenile - An animal less than one year of age.
lateral - Away from the midline of the body.
medial - Toward the midline of the body.
meninges - Referring to the membranes covering the brain and spinal cord.
pectoral - Coming from the chest.
post-mortem - Occurring after death.
putrefaction - The decomposition and breakdown of organic matter by bacteria and fungi.
sagittal - The median plane of the body, and any plane parallel to

Lesion-Interpretation/Evaluation

Lesions are defined as an injury to an organ or tissue.

Lesions are usually identified and characterized by their:

1. Location: area of body, organ, or tissue affected.

2. Distribution: focal, multifocal, diffuse, interstitial, locally extensive, embolic, lobar, etc.

3. Color: red, red-brown, brown, pale red, etc.

4. Shape: round, nodular, irregular, etc.

5. Surface Conformation: raised, level, depressed, umbilicated.

6. Size: dimensions in metric units.

7. Texture: firm, spongy, gritty, friable, etc.

General Supplies

Fixatives - 10% neutral buffered formalin
Labeling supplies
Sample containers
String for tying off intestinal loops
Pencils
Submission forms

Necropsy Tools

Scalpel blades and handles
Small and large dissection scissors
Long and short handled tweezers
Large, sharp skinning knife
Wetstone
Wire cutters for cutting ribs - (should be able to clean them easily)
Protective clothing - rubber gloves, aprons, etc.
Tape measure
Portable scale - (spring or platform scale)
Clipboard
Dissecting tray or board

PRE-NECROPSY EVALUATION

The animal's history will aide the diagnostician's evaluation of the animal. Record the exact location where the animal was found, including county, city, and street and the sex of the animal, the condition of the animal when found (fresh, fairly autolyzed, severely autolyzed), and whether it is alive or dead. If dead, also record the length, weight, estimated age, and nutritional condition. These measures are taken after euthanasia in live animals.

Before the necropsy begins, a thorough evaluation of symptoms and external body conditions should be recorded. If the animal is alive, its behavior should be noted and recorded. Central nervous system (CNS) involvement is indicated by abnormal behavior. Abnormal behavior can be described as circling endlessly, blindness, staggering, self mutilation, a stiffly gaited walk and/or daytime activity. Animals in a stuporous condition or exhibiting rapid, foamy jaw movement can also be considered to have CNS involvement.

Once you've established whether or not the animal has CNS involvement, you should examine its body condition. It is necessary to determine

DELIVERY, PACKAGING, AND MAILING INSTRUCTIONS

1. Intact Carcass

Necropsy (post-mortem examination) must be performed as soon as possible after death because tissue autolysis and putrefaction begin immediately. The chances of demonstrating or recovering disease-producing microorganisms and of recognizing significant tissue alterations diminish rapidly as post-mortem changes advance. Refrigeration of a carcass is helpful in slowing the advance of such changes, but it must be remembered that it requires many hours for the internal organs to reach an inhibitory temperature (~40°F). The time required for this depends on the size of the animal, amount of fat, and thickness of the hair coat. In large animals, it may take 48 hours or more. If refrigeration is not possible, a carcass should be doused with cold water immediately after death and placed in the coolest area possible until necropsy can be performed.

Carcasses may be frozen if a long delay (several days or more) before necropsy is anticipated. However, freezing causes the formation of ice crystals in the tissues which often precludes satisfactory interpretation

Many sick, dying, and dead raccoons are the result of a traumatic injury (hit by car); however, sick raccoons have a much greater risk of dying from traumatic injury.

Common raccoon diseases affecting the:

Nervous system (signs: abnormal behaviors, depressed, clumsy aggressive, circling, aimless wandering)

Rabies encephalitis - viral infection
Distemper encephalits - viral infection
Protozoal encephalits - parasitic (Sarcocystis sp) infection

Digestive system (signs: depressed, poor growth and weight gain, diarrhea, bloody diarrhea)

Parasitism - roundworms, tapeworms, and others
Parvoviral enteritis - viral infection

Respiratory system (signs: weakness, depressed, nasal discharge, cough)

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